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LOW-TEMPERATURE INJURY TO STORED SUGAR CANE [1]

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INTRODUCTION

Injury to sweet-potato roots and potato tubers caused by low temperatures has been reported. Harter and Whitney [2] found that sweet potatoes die if held at 10° C. for any considerable length of time. Peacock and Wright [3] found that immature potato stock was injured by storage at 32° to 36° F., and that in immature stock the areas around the eyes and the lenticels darkened and most of the buds were killed. The experiments outlined in this paper deal mostly with sugar cane under cold-storage conditions.

METHOD

The temperature of the cold-storage room was maintained a few degrees lower than the lowest temperature of the insulated chambers in which the cane was to be stored. The temperature of each chamber was regulated to within one-tenth of a degree of constancy by means of an electric heater regulated by thermostats, operating in conjunction with relays. The equipment was the same as that used by Lauritzen and Harter [4] for work on the storage of sweet potatoes. The humidity was maintained at 95 to 98 per cent by placing a large pan of water in each chamber. The air in each chamber was circulated continuously by means of a fan.

COLD-STORAGE EXPERIMENTS

The varieties of sugar cane for the first experiment, which was conducted at the Arlington Experiment Farm, Rosslyn, Va., were shipped from Cairo, Ga., November 15 and arrived in Washington D. C., November 19, 1923. The cane was stored at 10° C. from November 19 to December 6, 1923. On December 7 parts of this cane were stored in each of three chambers maintained at the different constant temperatures of 3°, 7°, and 12° C., respectively. Also some of the cane was stored at 0° C., the temperature of the cold-storage room. This temperature fluctuated about 1°. Part of the cane was treated with hot water [5] before storage, and part after storage and prior to

[1] Received for publication Sept. 28, 1928; issued March, 1929.

[2] HARTER, L. L., and WHITNEY, W. A. INFLUENCE OF SOIL TEMPERATURE AND SOIL MOISTURE ON THE INFECTION OF SWEET POTATOES BY THE BLACK-ROT FUNGUS. Jour. Agr. Research 32: 1153–1160, illus. 1926.

[3] PEACOCK, W. M., and WRIGHT, R. C. LOW TEMPERATURE INJURY TO POTATOES WHEN STORED SHORTLY AFTER HARVEST. Potato Assoc. Amer. Proc. (1926) 13: 99–101. [1927?]

[4] LAURITZEN, J. I., and HARTER, L. L. SPECIES OF RHIZOPUS RESPONSIBLE FOR THE DECAY OF SWEET POTATOES IN THE STORAGE HOUSE AND AT DIFFERENT TEMPERATURES IN INFECTION CHAMBERS. Jour. Agr. Research 24: 441–456, illus. 1923.

[5] BRANDES, E. W., and KLAPHAAK, P. J. GROWTH STIMULATION AND PEST AND DISEASE CONTROL BY HOT-WATER TREATMENT OF SUGAR CANE "SEED." La. Planter 71: 371–372, 392–394, 412, illus. 1923.

planting. On March 7, 1924, the cane was removed from storage and planted in the greenhouse. At the time of planting the eyes were counted, and on April 10 the germinated ones were counted, the results being expressed as germination percentages. The cane stored in the chambers had dried out somewhat, as indicated by the shriveling of the ends of the stalks. The results of the experiment are given in Table 1.

TABLE 1.—*Percentage germination of sugar cane, untreated or treated with hot water, and stored at different temperatures, 1923–24*

Variety and treatment	Percentage germination at storage temperatures of—		
	12° C.	7° C.	3° C.
Untreated:			
Cayana 10	5.0	17.6	0
Louisiana Purple	1.9	2.1	0
Treated with hot water in fall prior to storage:			
Cayana 10	30.7	33.3	0
Louisiana Purple	6.7	7.5	0
Treated with hot water in spring after storage:			
Cayana 10	9.7	18.1	0
Louisiana Purple	13.9	7.1	0

The cane stored at 3° C. did not germinate. The eyes appeared sound when planted, but storage at a continuous low temperature is apparently injurious. The cane stored at 7° germinated better than that stored at 12° except the Louisiana Purple treated with hot water in the spring prior to planting. In general, the cane treated with hot water before storage gave the best results, and that treated with hot water in the spring gave better results than the untreated.

Cane treated for 20 minutes with fungicides such as mercuric chloride (1 : 1,000) and formaldehyde (4 per cent) before storage gave results somewhat better than the untreated cane, but not so good as the cane treated with hot water. The use of fungicides on a large quantity of cane is expensive and is possible only when small lots are stored. On the other hand, the hot-water treatment is cheaper and more practical. The shipment of sugar cane from the cane-growing regions of the South to Washington, D. C. caused so much damage to the eyes that results may have been greatly influenced.

During succeeding years the experiments were carried on at Southdown Plantation, near Houma, La. The equipment used at Houma was similar to that used in 1923 at the Arlington farm, except that the chambers were larger. About 500 pounds of cane could be stored in each. The cane was stored in 6 chambers, 3 of which were operated at 6° C. and 3 at 9°. The cane was cut into convenient lengths and packed in moist cypress sawdust, which was kept moist by being sprayed with water at regular intervals. The varieties of cane used in the experiment were D–74 and D–95.[6] The cane, which was grown at the Quitman place on Bayou Grande Caillou, La., was hauled by truck to the place of storage. Here the leaves were removed, the cane was cut in half, and the tops were

[6] D denotes Demerara seedling.

placed in one pile and the butts in another. The cane was tied in bundles, each of which was labeled with an aluminum embossed label. Part of the cane was packed in sawdust and stored outside under an open shed to protect it from the weather, and part of it was windrowed in the field and used as a control. The various treatments of the cane and the results obtained are given in Table 2.

TABLE 2.—*Germination of sugar cane stored under different conditions, 1924–25*

Treatment	Variety	Portion of stalk	Temperature (° C.)	Number of eyes	Germination	
					Number	Per cent
Untreated	D–74	Base	6	135	13	9.6
		Top	6	106	8	7.5
	D–95	do	6	158	8	5.1
		Base	6	157	13	8.3
		Top	9	90	0	0
	D–74	Base	9	128	0	0
		do	9	167	4	2.4
		Top	9	98	1	1.0
Treated with hot water in fall prior to storage.	D–74	Base	9	168	7	4.2
	D–95	Top	9	100	0	0
	do	Base	9	96	5	5.2
	D–74	Top	9	133	8	6.0
Treated with cold water [a] in fall prior to storage.	D–95	Base	6	214	2	.9
	D–74	do	6	146	1	.7
	D–95	Top	6	149	6	4.0
	D–74	do	9	84	3	3.6
Stored under water	D–74		6	88	0	0
	D–95		6	93	0	0
Treated and stored outside; packed in sawdust. [b]	D–74	Whole		470	51	10.9
	D–95	do		298	15	5.0
Packed in sawdust	D–95	do		767	40	5.2
	D–74	do		104	9	8.7
Windrowed in the field	D–74			153	22	14.4
	D–95			173	42	24.3
Fall-planted cane	D–74			394	94	23.9
	D–95			406	96	23.6
	P. O. J. 234 [c]			341	114	33.4

[a] In water 72 hours before storage.
[b] Treated with 1:1,000 mercuric chloride for 20 minutes before storage.
[c] P. O. J. denotes Proefstation Oost Java seedling.

The data show that on the whole there is no significant difference between the keeping of tops and butts in cold storage, and that the cane treated with hot water, with cold water, and with mercuric chloride did not keep better than the untreated cane. The windrowed cane germinated better than that stored outside in sawdust or than the cold-storage cane, while the fall-planted cane germinated as well as or better than the windrowed. The experiment indicates that storage in the field is best for the varieties of cane used.

These experiments were made with canes that were passing out of cultivation and being replaced by the more vigorous Proefstation Oost Java (P. O. J.) varieties, which differ widely from the "noble" canes. They are vigorous canes, tolerant of or immune to mosaic, and develop a strong root system that is resistant to root rot.

In the fall of 1925 the chambers were removed from the cold-storage room. The room was operated at a temperature of 7° to 8° C. and divided into six compartments, each of which was large enough to hold about one-half ton of cane. Ventilation was provided by circulating the air in the room constantly by means of a fan and by installing a blower that introduced air at regular intervals. The cane was stored in moist cypress sawdust and in moist earth, which

were sprinkled regularly with water to keep them from drying out. Part of the cane was cut in half and the tops and the butts were stored separately. Some of the cane was treated with hot water before storage. Plots of the cane were planted in the fall, and the control material was windrowed in the field. The period of storage was from November 14, 1925, to March 4, 1926. At the time of planting, the eyes in each plot were counted, and on May 29 the germinated ones were counted. The following varieties were used in the experiment: D–74, P. O. J. 36, P. O. J. 213, P. O. J. 234, and Cayana. The results are given in Table 3.

TABLE 3.—*Percentage germination of seed sugar cane, untreated or treated with hot water, and stored at 7° to 8° C. under different conditions, 1925–26*

Parts of cane used, treatment and method of storage	Percentage germination of—					
	D–74	Cayana	P. O. J. 36	P. O. J. 213	P. O. J. 234	General average
Untreated before storage:						
Top	2.29	17.70	28.50	24.59	21.83	18.98
Butt	4.97	35.98	29.98	12.49	38.60	24.40
Stored in sawdust	3.98	14.29	26.72	18.63	28.66	18.46
Stored in dirt	4.37	39.95	31.59	12.59	26.35	22.97
Average	3.90	26.98	29.20	17.07	28.86	21.20
Hot water	4.41	41.82	21.33	0	38.09	21.13
Average treated and untreated cold-storage cane	4.00	29.95	27.62	13.66	30.71	21.19
Windrowed	1.50	26.62	13.64	15.97	9.23	13.39
Fall planted			[a] 27.00	[a] 31.36	[a] 28.90 [b] 28.47	28.93

[a] Cut A, light soil. [b] Cut C, heavy soil.

The data show that D–74 barely equals its performance of former years. The percentage of germination was very low. The P. O. J. varieties and Cayana gave results that were somewhat more promising, as their general average was from 13 to 31 per cent. This was better than the average of the windrowed cane, which was 9 to 26 per cent, but not so good as the fall-planted cane with an average of about 29 per cent. The experiment indicates that the cold storage of seed sugar cane is not practicable.

FREEZING POINT OF SUGAR-CANE JUICE

It is evident from Table 1 that continuous low temperatures are injurious to sugar cane, although the temperature may be a few degrees above the freezing point. The freezing point of sugar cane was known to be about 1° below the freezing point of pure water, but in order to get more definite knowledge, the freezing points of the cane juice and tissues were determined. The juice was extracted from the cane by means of a press. The juice extracted from cane frozen before extraction gave about the same results as that extracted from unfrozen cane, but the juice from frozen cane was a little easier to extract. Juice was taken from different parts of the cane and the freezing point was determined by means of a Beckmann thermometer. The results are shown in Table 4. The temperatures in the table are the true freezing points of the juice.

TABLE 4.—*Freezing points of juice extracted from different parts of sugar cane*

Variety	Place grown	Condition of cane	Freezing point (°C.) of juice from—			
			Base of stalk	Growing point	Leaves	Whole stalks
P. O. J. 213	Canal Point, Fla.	Healthy (7 months old).	−0.711	−0.522	−0.623	
Louisiana Purple	do	do	−.627	−.468	−.575	
Do	Greenhouse, Arlington Experiment Farm, Rosslyn, Va.	Mature, mosaic	−.689	[a] −.773		
Do	do	Young (5 feet tall)	−1.157	−.799	−.814	
Do [b]	do	Young (4¼ feet tall).	−.913	−.617	[c] −.674	
S. P. I 29109	do					−1.641
Louisiana Purple	do	Mature				−.720
P. O. J. 36	Houma, La	do	−1.101	−1.090		−1.205
P. O. J. 234	do	do				−1.318
P. O. J. 213	do	do				−1.258
P. O. J. 2725	do	do				−1.266

[a] Nodes near the top just out of the sheath.
[b] All nodes covered by the sheath.
[c] Leaf blade and sheath.

The freezing point of sugar-cane juice was found to vary from −0.468° C. to −1.090° in the topmost nodes; from −0.627° to −1.157° in the lower nodes; from −0.575° to −0.814° in the leaves, and from −0.72° to −1.641° in the whole stalk of mature cane. In the field the growing point and the younger leaves freeze before the older leaves and the lower portion of the stalk. The lower portion of the stalk withstands more cold than the eyes along the stalk. The lower eyes are usually a little more resistant to freezing than the growing tip. The amount of cold that they will stand depends to a certain extent upon the protection given by the leaves, and the condition of the soil. In Louisiana the cane on the light soil freezes at a lower temperature than that on the heavy black soils.

LOW-TEMPERATURE RESISTANCE OF SUGAR CANE

The length of time that sugar cane would stand a certain low temperature was determined in the following manner: One-eye cuttings of sugar cane were placed in a large test tube. The test tube was closed with a cork, and a thermometer was inserted. The cuttings were held in an ice box overnight and then in a cooling bath. When the desired temperature was reached, the tube containing the experimental material was placed in a slightly larger tube serving as a jacket to prevent direct contact between the experimental tube and the cooling bath, which was contained in a battery jar equipped with a ring-form stirrer. The equipment is the same as that used for freezing-point work. After treatment the cuttings were planted in the greenhouse, and germination counts were made about two weeks after planting. The results are given in Table 5.

TABLE 5.—*Low-temperature resistance of Louisiana Purple and P. O. J. 213 sugar cane as indicated by subsequent germination data*

[Planted June 3 and germination data taken June 16, 1924]

Variety	Temperature (° C.)	Time (minutes)	Germination[a]	Remarks
Louisiana Purple	−5	15	0	
		30	0	
		60	0	
		75	0	
Do	−2 to −2.5	15	+	Buds 4 inches long; roots large and vigorous.
		30	+	Buds beginning to grow; roots large.
		45	+	Do.
		60	+	Buds and roots medium, retarded.
		80	+	Buds and roots very small, retarded.
		90	0	Dead.
		105	0	Do.
		120	0	Do.
P. O. J. 213	−1.5	30	+	Buds and roots strong.
Louisiana Purple	−1.5	45	+	Do.
P. O. J. 213	−1.5	60	+	Do.
Louisiana Purple	−1.5	60	+	Buds and roots retarded.
P. O. J. 213	−1.5	75	+	Buds strong; no roots.
Louisiana Purple	−1.5	75	0	Buds dead; roots small.[b]
P. O. J. 213	−1.5	90	+	Buds 4 inches long; no roots.
Louisiana Purple	−1.5	90	0	Buds dead; roots about 1 inch long.[b]
P. O. J. 213	−1.5	105	+	Buds 3 inches long, no roots.
Louisiana Purple	−1.5	105	0	Buds dead; few short roots.[b]
P. O. J. 213	−1.5	120	0	Do.[b]
Louisiana Purple	−1.5	120	0	Dead.
Controls:				
P. O. J. 213				Growing much better than Louisiana Purple.
Louisiana Purple				Growing.

[a] + indicates germination.
[b] When only roots developed the cutting was considered dead, since it could not produce a plant.

The data show that the Louisiana Purple will not stand a temperature of −5° C. for 15 minutes, but that it will stand one of −2° to −2.5° for about 80 minutes. P. O. J. 213 will stand a temperature of −1.5° for about 105 minutes. The buds are usually killed before the roots, and the low temperature retards the development of both roots and buds. After exposure at −1.5° for about 60 minutes the buds were less retarded than the roots. When the buds are dead, however, the roots begin to develop quite soon. When only roots develop the cutting is considered dead because it can not produce a plant.

GROWTH OF FUNGI AT LOW TEMPERATURE

In the storage experiments the cane stored at different temperatures showed different degrees of deterioration, because of the activity of fungi. Most of the fungi that attack the dormant sugar-cane stalks or seed pieces are weak facultative parasites that normally live a saprophytic life and are always present in the soil of the cane fields. The most common are *Colletotrichum falcatum* Went., *Melanconium sacchari* Mass., *Cephalosporium sacchari* Butl., *Fusarium* sp., *Ceratostomella adiposum* (Butl.) Sartoris, Mucor, Rhizopus, and Penicillium. In fact, almost any fungus will grow on the dormant sugar-cane stalk. The cane stored at 12° C. was rotted extensively. Several fungi were isolated from the cane in storage, and their rates of growth at different temperatures were determined. All the temperatures were constant within one-tenth degree Centigrade except "0," which was the temperature of the cold-storage room and fluctuated about 1°, and "about 20° C.," which was the laboratory temperature and ranged

from 18° to 21°. The fungi were planted on sugar-cane agar and held at a constant temperature. The rate of growth is expressed in the number of days required for the fungus to cover an area of 63.6 sq. cm. This is an arbitrary measurement and does not take into account the different growth habits of the organisms. The results are given in Table 6.

TABLE 6.—*Growth rates of various fungi on sugar-cane agar*

[0 indicates no growth when the experiment was terminated, and the footnotes indicate fractions of 63.6 sq. cm. when the whole area was not covered]

Fungus and method of storage	Days required to cover an area of 63.6 sq. cm. at temperatures of—				
	About 20° C.	12° C.	7° C.	3° C.	0° C.
Stored immediately at experimental temperatures:					
Rhizopus sp.	2	7	34	0	0
Botrytis sp.	4	4	8	13	28
Cephalosporium sacchari	5	14	29	0	0
Colletotrichum falcatum	5	21	27	0	0
Fusarium sp.	5	16	26	[c] 19	
Penicillium sp.	2	14	0	0	0
Aspergillus sp.	4	0	0	0	0
Basisporium galorum	2	7	[b] 13		
Colletotrichum falcatum	5	14	26	[b] 19	
Grown for 36 hours at 20° C. before being stored at experimental temperatures:					
Rhizopus sp.		8		[c] 57	
Botrytis sp.		5		17	
Cephalosporium sacchari		8		[d] 57	
Colletotrichum falcatum		8		[d] 57	
Penicillium sp.		8		[e] 57	
Colletotrichum falcatum		8		[c] 57	

[a] Only one-tenth of area covered.
[b] Only one-fifth of area covered.
[c] Only one-third of area covered.
[d] Only one-fourth of area covered.
[e] Only one-half of area covered.

The data show that all the fungi except Aspergillus grow fairly well at 12° C. and that most of them grow well at 7°. *Botrytis* sp. grows well at 3°, and *Colletotrichum falcatum* grows slowly at this temperature. The only fungus to grow at 0° was Botrytis. At room temperature (about 20°) the more rapid growers developed at two-and-a half times the rate of the slower ones, but this same ratio is not maintained at the lower temperatures. If the fungi are given a start of 36 hours at room temperature before they are placed at 12°, some of them develop faster than they would if started at the lower temperature. On the other hand, if the cultures are grown at room temperature for 36 hours, and then placed at 3°, the growth of the fungi is either retarded or checked.

The rate of growth of the fungi on sugar-cane cuttings was also determined. Two-eye cuttings of cane were sterilized in mercuric chloride (1 : 1,000 solution) for 20 minutes and rinsed in sterile water. The cuttings were placed in large sterile test tubes, inoculated with the different fungi, and placed at the various temperatures. The cuttings were carefully chosen with respect to size and length, especially the length of the cut internode to the first node. The rate of growth is given in Table 7. The controls were treated in the same manner except that they were not inoculated with fungi.

TABLE 7.—*Growth rates of fungi on sugar-cane cuttings, April 19 to May 20*

Fungus	Color of growth	Growth (in centimeters) from point of infection at temperatures of—				
		About 20° C.	12° C.	7° C.	3° C.	0° C.
Rhizopus sp.	Reddish brown	7.0	2	Slight.	0	0
Botrytis sp.	Slightly red	2.5	[a] 15	[b] 6	[c] 4.5	Slight.
Cephalosporium sacchari	Red	6.0	35	19	0	0
Colletotrichum falcatum	do	23.0	7	21	Slight.	0
	do	8.0	14	22	do	0
Fusarium sp.	do	15.0	4	[c] 6	0	0
Penicillium sp.		4.0	[d]	16	0	0
Aspergillus sp.	Red	7.0	1	[d]	0	0
Controls [e]		0	0	0	0	0

[a] 2 nodes.
[b] 1 node.
[c] Mycelium abundant.
[d] Very slight.
[e] No infection, but cut ends red for a few centimeters.

The eyes of nearly all of the cuttings germinated while in the tubes. The results are not entirely conclusive, but they do show that most of the fungi grow rather well at 12° C.; two of them grow very well, and two rather well, at 7°. There is little or no growth at 3°, except of Botrytis, which seems to be inhibited very little by low temperatures, developing at a good rate at 0°.

It is clear from Tables 5 to 7 that the range of temperature at which sugar cane can be stored is very small. At temperatures below 7° C., where the growth of fungi is inhibited, the cane is injured to such an extent that the percentage of germination is very low. At higher temperatures the fungi develop so rapidly that a large part of the cane is rotted. The experiments show that a temperature of about 8° to 10° is best for the storage of sugar cane.

It is doubtful whether cold storage would be practicable even if 90 per cent of the eyes were carried over the period of storage in a healthy condition, because the manner of planting and the growing conditions in the Sugar Belt of the United States are such that not more than 25 to 35 per cent of the eyes would germinate and reach maturity in the sense of developing into full-sized plants. The first difficulty in storage is that there is little healthy cane. About 25 per cent of the eyes are destroyed by borers, and the burrows made by the borers become infested with fungi. Starting with such cane, and knowing the limited cold that the cane will stand, it is evident that one can hardly devise conditions of cold storage that will keep all eyes sound.

Cold storage may have a practical application in the shipment of small quantities of seed cane that are to be in transit a long time. In such cases healthy stalks or cuttings could be selected, sterilized, and stored at temperatures of 8° to 10° C. If the cane is packed in some material that will maintain the moisture content of the cane, it could be in transit three or four months without losing its power to germinate. By referring to Table 3, it will be noticed that there was a germination of 29.2 per cent in P. O. J. 36 after storage at 7° to 8° C. for about three months, and that in P. O. J. 234, when treated with hot-water, there was a germination of 38.09 per cent. This cane, which was not selected was bored and infested

with fungi when stored. Had sound cuttings been chosen and had sterile packing material been used the percentage of germination would no doubt have been very much greater.

SUMMARY

The best temperature for the storage of sugar cane is 8° to 10° C. The cane should be packed in some material that will maintain its moisture content.

The cold storage of sugar-cane seed as a regular plantation practice is not practicable. At a continuous temperature of 3° C. the seed cane is injured to such an extent that it will not germinate, although it may appear sound and fresh. Most of the fungi that cause damage to seed cane will grow well at 12° and some of them at 7°. At a temperature as low as 6° there is development of the roots and buds of the sugar cane.

The freezing point of the juice of sugar cane is about −1° C.

Seed cuttings of Louisiana Purple will not germinate after exposure to a temperature of −2° to −5° C. for more than 80 minutes. P. O. J. 213 germinated after exposure to a temperature of −1.5° for 105 minutes.

INHERITANCE OF YIELD AND PROTEIN CONTENT IN CROSSES OF MARQUIS AND KOTA SPRING WHEATS GROWN IN MONTANA[1]

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IMPROVEMENT IN YIELD AND PROTEIN CONTENT OF SPRING WHEAT DESIRED

More profitable production of hard red spring wheat is dependent upon increased acre yields, together with higher protein content of the grain. At present Marquis is the leading commercial variety of spring wheat. Under favorable conditions it is a very high yielding wheat, but under unfavorable conditions caused by drought or stem rust, such as frequently occur in the Dakotas, Kota usually outyields Marquis. This fact, together with the higher crude-protein content of the grain of Kota, made it by 1924 the second most widely grown variety of hard red spring wheat. Marquis is awnleted and Kota is awned. The awned segregates from crosses of these varieties have been higher yielding than the awnleted in Minnesota and North Dakota. The effect of awns and inheritance of yield and protein content in Marquis and Kota crosses under favorable Montana conditions is herein shown, as the results differ from those obtained in Minnesota and North Dakota under less favorable conditions.

MATERIAL AND METHODS

Much success has been obtained with crosses of Marquis and Kota wheats in North Dakota by Waldron (*10*)[2] and in Minnesota by Hayes and Aamodt (*9*). From this cross has been developed the Ceres variety, which in 1928 was grown on at least 40,000 acres. Even more promising selections from this cross are now being tested at the North Dakota and Minnesota agricultural experiment stations. All of the most promising selections developed in these States are awned. None of these has shown to as great an advantage in Montana as in the other spring-wheat States. Because of this, and to meet the popular demand in Montana for an awnless spring wheat, the writers projected the present study. It was designed to determine the relation of the length of awns to yield and protein content of the kernel under favorable conditions in Montana, and to develop awnleted strains which will be better adapted for Montana than the parent varieties and the awned strains developed from them in other States.

PARENT MATERIAL

The awnleted Marquis and awned Kota varieties have been included in the spring wheat varietal plots at the Bozeman, Moccasin, and Havre stations in Montana for several years. Milling and bak-

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[2] Reference is made by number (italic) to Literature cited, p. 217.

ing experiments, including crude-protein tests, have been made on wheat samples from these experiments.

Yield per Acre

The annual and average yields from Marquis and Kota wheats obtained from the plot experiments at the experiment stations in Montana from 1921 to 1925 are given in Table 1.

TABLE 1.—*Yields of Marquis and Kota wheats grown in plot experiments at Bozeman, Moccasin, and Havre, Mont., during the five years 1921–1925*

Station and variety	Yield per acre (bushels)—						Percentage of Marquis
	1921	1922	1923	1924	1925	Average	
Bozeman:							
Marquis	42.7	64.3	57.2	36.5	47.4	49.6	100.0
Kota	40.7	57.0	40.1	36.4	43.9	43.6	87.9
Moccasin:							
Marquis	28.3	27.3	24.6	32.0	22.4	26.9	100.0
Kota	28.3	26.5	23.8	24.7	23.1	25.3	94.1
Havre:							
Marquis	11.7	5.8	20.3	12.3	16.4	13.3	100.0
Kota	15.2	3.5	21.7	12.7	18.1	14.2	106.8
Average (3 stations):							
Marquis	27.6	32.5	34.0	26.9	28.7	29.9	100.0
Kota	28.1	29.0	28.5	24.6	28.4	27.7	92.6

The data show that, under the more favorable conditions at Bozeman, Marquis exceeds Kota in average acre yield by 6 bushels, or 12.1 per cent. Under less favorable conditions at Moccasin, Marquis exceeds Kota in average yield by only 1.6 bushels, or 5.9 per cent. At Havre, under conditions unfavorable or marginal for profitable wheat production, Kota is the higher yielding variety, exceeding Marquis by 0.9 bushel, or 6.8 per cent. The average difference for the 15 station years at the 3 stations is 2.2 bushels, or 7.4 per cent, in favor of Marquis.

These yields, which were available at the time this breeding study was begun, show that under increasingly favorable conditions the awnleted Marquis has a corresponding advantage over the awned Kota.

It appeared desirable, therefore, to determine, under the most favorable conditions, whether the awns of Kota, which had been found to be an advantage in North Dakota and Minnesota (*2*, *8*), have any effect on yields in Montana.

Crude-Protien Content

The results of crude-protein tests made on samples of Kota and Marquis from the plot experiments in connection with milling and baking experiments are given in Table 2.

TABLE 2.—*Crude-protein content of Marquis and Kota wheats grown in plot experiments at Bozeman, Moccasin, and Havre, Mont., during the five years 1921–1925*

Station and variety	Crude-protein content (per cent)—						Percentage of Marquis
	1921	1922	1923	1924	1925	Average	
Bozeman:							
Marquis	13.1	10.5	13.4	10.7	11.8	11.9	100.0
Kota	16.1	12.9	16.2	12.1	13.9	14.2	119.3
Moccasin:							
Marquis	14.5	15.0	13.0	14.5	17.0	14.8	100.0
Kota	14.8	15.7	14.3	15.9	17.7	15.7	106.1
Havre:							
Marquis	17.6	15.5	13.8	12.9	15.0	15.0	100.0
Kota	18.2	14.7	14.6	13.9	16.2	15.5	103.3
Average (3 stations):							
Marquis	15.1	13.7	13.4	12.7	14.6	13.9	100.0
Kota	16.4	14.4	15.0	14.0	15.9	15.1	108.6

The data show Kota to exceed Marquis at all stations and in all years, except at Havre in 1922. The largest average difference is at Bozeman, where Kota had 2.3 per cent more protein than Marquis, an increase of 19.3 per cent. Under less favorable conditions at Moccasin and Havre the average differences decreased. The average for the 15 station years at the 3 stations shows a difference of 1.2 per cent protein, an increase for Kota of 8.6 per cent over Marquis. This is a somewhat larger difference than was found by Clark and Shollenberger (*6*) from 100 comparable samples, obtained over a period of 6 years from experiment stations in 11 States. The average difference there shown was 0.77 ± 0.21 per cent in favor of Kota.

It seemed desirable to determine in connection with yield whether there was any effect of awns on the crude-protein content of hybrid plants and strains under favorable conditions in Montana.

HYBRID MATERIAL

The crosses, Marquis × Kota and its reciprocal, were made by V. H. Florell in experiments cooperative with the California Agricultural Experiment Station, at Davis, Calif., in 1924. Some F_1 plants were grown in a greenhouse at the Arlington Experiment Farm, Rosslyn, Va., in the winter of 1924–25. In 1925 F_1 and F_2 plants were grown at the Montana Agricultural Experiment Station, Bozeman, Mont. In 1926 F_2 and F_3 progenies and in 1927 F_3 and F_4 progenies were grown at Bozeman, Mont. The more advanced material obtained from growing the F_1 plants in the greenhouse was not studied for inheritance of characters, but awnleted selections were made for practical plant-breeding purposes.

SEGREGATION OF CHARACTERS

The inheritance of dwarfness, awnedness, yield, and protein content was studied on F_2 plants grown in 1926 and F_3 strains from them grown in 1927.

In both 1926 and 1927 the hybrid and parent check plants were grown from seeds spaced 3 inches apart in rod rows 1 foot apart. In 1927 the parent checks occupied every tenth row, after single rows of nine F_3 strains arranged in the random order of harvesting in the F_2 generation.

DWARFNESS

The F_1 plants of the crosses Marquis×Kota and its reciprocal were normal in stature, but in F_2 dwarf plants were obtained. Table 3 presents the data for normals and dwarfs in the F_2 generation grown at Bozeman, Mont., in 1926. In a total of 495 F_2 plants grown, 88 were dwarf. All F_1 families of both reciprocal crosses segregated in very close agreement with a calculated 13:3 ratio.

TABLE 3.—*Segregation of 495 F_2 plants of Marquis×Kota and reciprocal crosses into two classes, normal and dwarf, when grown at Bozeman, Mont., in 1926*

Cross	Number of F_1 families	Number of F_2 plants			Deviation from 13:3 ratio	Probable error	Deviation÷probable error
		Normal	Dwarf	Total			
Marquis×Kota	4	199	41	240	4.0	4.1	1.0
Kota×Marquis	4	208	47	255	.8	4.2	.2
Total	8	407	88	495	4.8	5.9	.8

Additional data were obtained on dwarf and normal plants in the F_3 generation. Of normal plants of the Kota×Marquis cross 195 were continued in the F_3 generation. These were all of the plants of this cross from which there was sufficient seed. Only normal plants were used, because none of the dwarf F_2 plants matured seed. The results are given in Table 4.

TABLE 4.—*Breeding behavior of 195 F_3 strains grown from normal F_2 plants of Kota×Marquis crosses at Bozeman, Mont., in 1927*

Ratio	Number of F_3 strains		
	Normals breeding true	Segregating dwarfs	Total
Obtained	106	89	195
Calculated on 7:6 ratio	105	90	195

The data presented in Table 4 show that of the 195 F_3 families 106 bred true for normal plants, and 89 segregated for dwarf, which was very close to a calculated 7:6 ratio, the deviation being 1 ± 4.7. The F_2 and F_3 data, therefore, fit very well the theoretical 13:3 ratio.

A satisfactory interpretation of the above data can be made on a genetic basis by assuming a factor pair DD for dwarf plants and a factor pair NN for normal plants, which inhibits the action of D. As Clark (*2*) obtained no dwarfs in Kota×Hard Federation crosses, and Clark and Hooker (*5*) found segregation for dwarf plants in Marquis×Hard Federation crosses, the formula for Kota could be DDNN and that for Marquis ddnn, or vice versa. The following formulas may represent the F_2 phenotypes and genotypes and their breeding behavior in the F_3 generation:

F_2 generation	F_3 generation
Normal:	
1 DDNN	Breeding true.
2 DdNN	
1 ddNN	
2 ddNn	
1 ddnn	
7	
4 DdNn	Segregating in a 13 : 3 ratio.
2 DDNn	Segregating in a 3 : 1 ratio.
6	
Dwarf:	
2 Ddnn	Segregating in a 3 : 1 ratio.
1 DDnn	Breeding true.

The normal plants continued in the F_3 thus will have seven parts breeding true for normals and six parts segregating for dwarfs. In this study no attempt was made to separate the segregating families according to whether they were breeding in a 13:3 or a 3:1 ratio.

It is concluded that in this case Marquis and Kota differed by two genetic factors for the dwarf character. This agrees with the conclusions of Goulden (7). As he found back crosses of true breeding dwarfs to the parents produced in the F_1 generation only dwarfs in the cross of dwarf × Marquis, and only normals in the cross of dwarf × Kota, the formula for Kota would be DDNN and that Marquis ddnn.

AWNEDNESS

The spikes of F_1 plants of the Marquis × Kota and reciprocal crosses were strongly awnleted in contrast to Marquis, which is weakly awnleted, and Kota, which is awned. This showed imperfect dominance of awnletedness. In the F_2 material grown at Bozeman, Mont., in 1926, an attempt was made to separate the plants into three awnedness groups, although there was but a small difference between the strongly awnleted and the weakly awnleted classes. The F_2 data are given in Table 5.

TABLE 5.—*Segregation of 407 F_2 plants of the Marquis × Kota and reciprocal crosses into three classes for awnedness, when grown at Bozeman, Mont., in 1926*

Awnedness	Kota × Marquis	Marquis × Kota	Total	Calculated on 1:2:1 ratio *	Deviation from a 3:1 ratio	Probable error
Awnleted:						
Weakly	52	31	83	102		
Strongly	111	120	231	203		
Total	163	151	314	305	9	5.9
Awned	45	48	93	102	9	5.9

* $P=0.017$.

The data for the total F_2 are not close to a calculated 1:2:1 ratio, $P=0.017$, but are a good fit to a 3:1 ratio when the two awnleted groups are combined and compared with the awned. It will be seen that in the Kota × Marquis crosses a better fit to a 1:2:1 ratio was obtained than in the Marquis × Kota crosses. It appears that in

classifying the F_2 plants too many of the weakly awnleted plants were placed with the strongly awnleted.

Most of the Kota×Marquis material was continued in the F_3, and the data are given in Table 6.

TABLE 6.—*Breeding behavior of 195 F_3 strains from Kota×Marquis wheat crosses of the three awnedness groups grown at Bozeman, Mont., in 1927*

F_2 classes and groups	Number of F_3 strains—			Total
	Awnleted	Segregating	Awned	
Awnleted:				
Weakly—				
Number	44	4		48
Percentage	91.7	8.3		
Strongly—				
Number	13	94		107
Percentage	12.1	87.9		
Awned:				
Number			40	40
Percentage			100	
Total number	57	98	40	195

It is shown in Table 6 that all of the awned F_2 plants bred true in the F_3 generation and that none of those classed as awnleted were awned. The weakly awnleted plants bred true to the extent of 91.7 per cent, whereas 87.9 per cent of the strongly awnleted plants segregated. This shows that there was considerable overlapping in the awnleted groups and that in the classification of the F_2 some heterozygous plants were classed as weakly awnleted.

Correcting the total F_2 data given in Table 5 on the basis of the breeding behavior of the F_3 strains, as shown in Table 6, the numbers would be 104 : 210 : 93, and the calculated numbers on the theoretical ratio of 1 : 2 : 1 would be 101.75 : 203.50 : 101.75. This shows a very close agreement, $P = 0.604$. This brings out the fact that the awned and awnleted classes can be accurately classified, but that in this cross it was difficult to distinguish accurately between the weakly awnleted homozygous and the strongly awnleted heterozygous groups without growing and studying an F_3 population.

From the data presented it is clearly shown that only a single genetic factor difference for awnedness is involved in Kota×Marquis crosses. This is in agreement with the early studies of Biffen (*1*) and others and with F_2 results shown by Hayes and Aamodt (*9*) for the same cross.

YIELD

Yield is largely the result of the interaction of environment and the physiological and morphological characters of the plant. Morphological characters of the wheat plant in their relation to yield have not been extensively studied. From contrasting awnedness classes in wheat crosses, Hayes (*8*) in Minnesota and Clark (*2*) in both Minnesota and North Dakota found yield to increase with the length of awns in hybrid segregates. Under the conditions at Davis, Calif., Clark, Florell, and Hooker (*4*) found that there was but a slight tendency for yield to be increased with increasing length of awns in hybrid plants and strains.

In the present work, plant yields of the parents and of hybrids in the F_2 and F_3 generations were determined for the purpose of studying the inheritance of yield and the relation of the degree of awnedness to yield under the favorable conditions at Bozeman, Mont.

The F_2 data are given in Table 7. It will be seen that Marquis had an average plant yield of 7.21 ± 0.12 and Kota had a yield of 6.73 ± 0.14. gm. The difference is 0.48 ± 0.18 and is not significant in the light of its probable error. The mean yield of the hybrids is intermediate between those of the two parents, although there are hybrid plants which exceed the upper yield limit of either parent. The coefficient of variability shows that the F_2 plant yields were significantly more variable than the plant yields of either parent. This is in accord with theoretical expectation for quantitative characters. The data on the F_3, in comparison with those from the parents, are given in Table 8.

TABLE 7.—*Plant yields of Marquis and Kota parents, total F_2 hybrids, and awnleted and awned hybrids, by 2-gm. frequency classes, at Bozeman, Mont., in 1926*

Yield per plant in grams	Number of plants of—				
	Parents and hybrids			F_3 hybrids by awnedness classes	
	Marquis	Total F_2 hybrids	Kota	Awnleted	Awned
1	2	37	6	20	17
3	18	58	28	41	17
5	42	75	53	57	18
7	57	72	46	64	8
9	53	61	36	49	12
11	18	40	18	35	5
13	5	28	7	21	7
15		10	2	8	2
17		3		3	0
19		2		1	1
Total plants	195	386	196	299	87
Mean yield	7.21±0.12	6.95±0.13	6.73±0.14	7.24±0.14	5.94±0.30
Standard deviation	2.48± .08	3.84± .09	2.92± .10	3.68± .10	4.17± .21
Coefficient of variation	34.40±1.31	55.25±1.70	43.39±1.73	50.83±1.72	70.20±5.06

TABLE 8.—*Average plant yields of Marquis and Kota parents, total F_3 hybrids, and the awnleted, segregating, and awned hybrids, by 1.5-gm. frequency classes, at Bozeman, Mont., in 1927*

Yield per plant in grams	Number of plants of—					
	Parents and hybrids			F_3 hybrids by awnedness groups		
	Marquis	Total F_2 hybrids	Kota	Awnleted	Segregating	Awned
0.8		1			1	
2.3		1			0	1
3.8		7			3	4
5.3	2	26		5	13	8
6.8	5	56	4	14	30	12
8.3	5	56	4	24	20	12
9.8		32	2	11	19	2
11.3		12		3	8	1
12.8		3	1		3	
14.3		1			1	
Total plants	12	195	11	57	98	40
Mean yield	7.18±0.21	7.77±0.10	8.44±0.36	8.12±0.13	7.96±0.15	6.80±0.20
Standard deviation	1.06± .15	2.05± .07	1.75± .25	1.49± .09	2.26± .11	1.87± .14
Coefficient of variation	15.04±2.12	26.38± .96	20.73±3.11	18.35±1.20	28.39±1.47	27.50±2.22

A total of 195 F_3 hybrid strains from normal F_2 plants was continued in 1927. Because dwarf plants were present in some of the hybrid rows it seemed best, for reasons stated later, to present the average yield per row on a plant basis. In 1927 the Kota check rows had a higher average plant yield than the Marquis checks. The difference of 1.26 ± 0.42 gm. is three times its probable error, giving odds of about 22 : 1 that the difference is not due to chance. This is the reverse of the results in 1926. The F_3 hybrid rows again were intermediate in yield between the parent checks, and there were several strains that were lower and one that was higher in yield than the extremes of both parents.

Relation of Awnedness to Yield

In the F_2 generation the average yield of the awnleted hybrid plants exceeded by 1.30 ± 0.33 gm. the average yield of the awned plants. There was no significant difference in the variability of the two classes. The difference in yield is due in part to greater shattering among the awned plants. The frequency distribution of the awned plants shows that there is a bunching of the plants in the lower yield classes and that the curve is not normal, as it was for the awnleted class. This apparently was caused by the shattering which occurred.

The data in Table 8 show the average plant yields of F_3 strains in the awnleted, segregating, and awned groups. As in the F_2, the awnleted exceeded the awned strains in yield, even though the awned Kota parent outyielded the awnleted Marquis parent. The difference in average yield between the awnleted and awned F_3 strains is 1.32 ± 0.24 gm., which is statistically significant. As in the F_2 generation, shattering occurred, and again it was much worse in the awned than in the awnleted strains. Shattering estimates taken on a row basis at harvest time show that the awnleted strains shattered 8.25 per cent, whereas the awned rows shattered 14.38 per cent. This difference in shattering tends to increase the difference in yield between the two awnedness groups. Had no shattering occurred, the awnleted strains apparently still would have outyielded the awned, but the difference would not have been so great.

Relation of Presence of Dwarf Plants to Yield

In the F_3 generation there were 106 hybrid strains which had no dwarf plants. These yielded on an average 41.89 ± 0.64 bushels per acre. There were 89 strains which had contained dwarfs, and these yielded 35.31 ± 0.65 bushels per acre. The difference of 6.58 ± 0.91 bushels in favor of the rows without dwarfs is 7.2 times the probable error and is very significant.

In order to discover whether the presence of dwarf plants in the row would permit an increase in the yield per normal plant, the average yield per plant in the row was determined. It was found that the average yield per normal plant in rows without dwarfs was 7.65 ± 0.13 gm., while in rows with dwarfs the average yield per normal plant was 7.91 ± 0.15 gm. The difference of 0.26 ± 0.20 gm. is not significant, as it is only 1.3 times the probable error. This brings out clearly the fact that the reduction in yield is due to the dwarf plants failing to head, as they appear to use the available moisture and plant food to such an extent that the normal plants are unable to produce higher yields.

CRUDE-PROTEIN CONTENT

The crude-protein content of wheat is of economic importance, as premiums are paid for high-protein wheat. The value of wheat for bread making is improved by increasing the protein content.

Clark and Hooker (*5*) have reported the segregation and inheritance of crude-protein content in hybrids between Marquis and Hard Federation wheats under Montana conditions. In that cross Marquis was the high-protein parent. In the present study of inheritance in crosses between Marquis and Kota wheats, Marquis is the low-protein parent.

The crude-protein content of the grain from F_2 plants and parents and of the bulk grain from nursery rows of F_3 strains and parents was determined [3] from a 2-gm. sample of whole kernels. Only single determinations were made because of the limited quantities of grain from single plants. The F_2 data are given in Table 9.

TABLE 9.—*Crude-protein content, by 1 per cent frequency classes, of the Marquis and Kota parents, the total population of F_2 hybrid plants, and the awnleted and awned hybrids, grown at Bozeman, Mont., in 1926*

Crude-protein content (per cent)	Number of plants of—				
	Parents and hybrids			F_2 hybrids by awnedness classes	
	Marquis	Total F_2 hybrid population	Kota	Awnleted	Awned
10.5		3		3	
11.5		13	1	9	4
12.5	10	26	11	22	4
13.5	24	31	20	24	7
14.5	58	34	29	26	8
15.5	35	39	21	31	8
16.5	6	26	38	21	5
17.5		5	14	3	2
18.5		3	6	3	
19.5		1	1	1	
Total plants	133	181	141	143	38
Mean protein	14.52±0.05	14.44±0.09	15.37±0.09	14.44±0.10	14.42±0.18
Standard deviation	.96± .04	1.76± .06	1.65± .07	1.78± .07	1.66± .13
Coefficient of variation	6.61± .27	12.19± .44	10.75± .44	12.33± .50	11.51± .90

Protein data are available from only 181 plants, because in some cases all of the grain was needed for seeding the F_3 rows. Kota had an average protein content of 15.37 ± 0.09 per cent, whereas the Marquis protein content averaged 14.52 ± 0.05 per cent. The average protein content of the F_2 plants was slightly, but not significantly, less than that of Marquis. It will be noticed that Marquis was much less variable than Kota in protein content. The F_2 hybrid plants were much more variable than Marquis and slightly more variable even than Kota. This greater variability of the hybrids over the parents has not been obtained in other studies (*3*, *4*, *5*) for segregation of protein content in wheat crosses.

The F_3 data on hybrid strains and parent check rows in 1927 are shown in Table 10.

[3] The crude-protein tests were made in the nitrogen laboratory of the Bureau of Chemistry and Soils by H. M. Joslin under a cooperative agreement with the Office of Cereal Crops and Diseases, Bureau of Plant Industry.

TABLE 10.—*Crude-protein content, by 0.5 per cent frequency classes, of the Marquis and Kota parents, total population of F_3 hybrid strains, and the awnleted, segregating, and awned hybrids, grown at Bozeman, Mont., in 1927*

Crude-protein content (per cent)	Number of plants of—					
	Parents and hybrids			F_3 hybrids by awnedness groups		
	Marquis	Total F_3 hybrid population	Kota	Awnleted	Segregating	Awned
13.3		2		1	1	
13.8		7		2	5	
14.3		36		16	19	1
14.8	8	29		9	18	2
15.3	2	52	1	16	22	14
15.8	1	49	4	12	25	12
16.3	1	17	4	1	6	10
16.8		3	2		2	1
Total plants	12	195	11	57	98	40
Mean protein	15.09±0.09	15.20±0.03	16.12±0.09	14.98±0.06	15.14±0.05	15.69±0.06
Standard deviation	.48± .07	.72± .02	.44± .06	.66± .04	.73± .04	.52± .04
Coefficient of variation	3.18± .44	4.74± .16	2.73± .39	4.41± .28	4.82± .23	3.31± .25

These data show that Kota averages 1.03 ± 0.13 per cent more protein than Marquis. The mean of all F_3 rows is intermediate between those of the two parents. No strains were obtained showing a higher protein content than the highest Kota check row, but several strains had a lower protein content than the lowest Marquis check row. The hybrids again were more variable in protein content than either parent.

RELATION OF AWNEDNESS TO PROTEIN CONTENT

Table 9 presents the data for protein content of the awnleted and awned classes of F_2 plants. Although the awnleted group showed a greater range of protein content, the averages of the two groups were practically equal.

The F_3 data, as given in Table 10, show that the awned group is significantly higher in protein content than the awnleted, the difference, 0.71 ± 0.08 per cent, being nearly nine times its probable error. The segregating group averages intermediate between the awnleted and awned groups. These data are in general agreement with those for yield, as protein content and yield are usually negatively associated; i. e., as yield increases protein content decreases.

It may be concluded, therefore, that in the Kota × Marquis cross, grown under favorable conditions at Bozeman, Mont., awnleted selections average higher in yield but lower in protein content than do awned selections. While there may be exceptions, this would appear to be the reaction of awnedness under favorable Montana conditions as compared with the opposite reaction under less favorable conditions, as has been found in North Dakota and Minnesota.

RELATION OF PRESENCE OF DWARF PLANTS TO PROTEIN CONTENT

In order to determine what effect, if any, the occurrence of dwarf plants had on protein content of normal plants, the average protein content of the strains with dwarfs and without dwarfs was obtained. There were 106 F_3 strains which had no dwarfs and 89 strains with dwarfs. It was found that the strains with dwarfs had an average

protein content of 15.15 ± 0.05 per cent and those without dwarfs had 15.24 ± 0.05 per cent. The difference is only 0.09 ± 0.07 per cent, showing that the presence of dwarfs had no influence on protein content.

CORRELATION OF CHARACTERS

The correlated inheritance of the quantitative characters, the yield and protein content, and the relation between these characters has been studied.

YIELD AND PROTEIN

Data are available on 181 F_2 plants for both plant yield and crude-protein content. The correlation coefficient obtained for yield and protein content on these F_2 plants was negative, $r = -0.124 \pm 0.049$. This coefficient is not large and, as it is only 2.5 times its error, is not significant. This indicates that there was no important relation between the yield and protein content in the F_2 plants of the Kota $\times$ Marquis crosses grown at Bozeman, Mont., in 1926.

In 1927 the correlation between the yield and protein content of 195 F_3 strains also was negative, $r = -0.281 \pm 0.044$. This coefficient is hardly important from a plant-breeding standpoint, although it is statistically significant.

It may be concluded that under these Bozeman conditions there was a slight tendency for yield and protein content to be negatively correlated; i. e., as yield increases, protein content decreases, but that it was not of such magnitude as to nullify the effect of selecting for quality on the basis of protein content.

YIELD OF F_2 AND F_3

The coefficient of correlation obtained for yield of 181 F_2 plants with that of the average yield per plant of F_3 rows was positive, $r = 0.186 \pm 0.047$. This is not an important correlation, although mathematically it is significant.

PROTEIN OF F_2 AND F_3

The coefficient of correlation obtained for protein content of 173 F_2 plants and that of the average of F_3 strains was positive, $r = 0.413 \pm 0.043$. This correlation is both significant and important. The correlation surface is given in Table 11.

TABLE 11.—*Correlation between crude-protein content of F_2 plants and that of F_3 strains of Kota $\times$ Marquis wheat crosses grown at Bozeman, Mont., in 1926 and 1927*

Crude-protein content of F_2 plants (per cent)	Distribution of F_3 strains by classes for crude-protein content [a] as indicated								
	13.3	13.8	14.3	14.8	15.3	15.8	16.3	16.8	Total
10.5		1	1	1					3
11.5		2	3	3	4	1			13
12.5	1	2	3	2	7	7		2	24
13.5			5	3	10	8	4		30
14.5	1		7	3	14	6	3		34
15.5			7	9	9	9	2	1	37
16.5			3	4	3	11	2		23
17.5						3	2		5
18.5			2		1				3
19.5							1		1
Total plants	2	5	31	25	48	45	14	3	173

[a] $r = 0.413 \pm 0.043$.

The distribution of the data in Table 11 illustrates the relationship which exists between the protein content of F_2 plants and that of F_3 strains. It indicates that in breeding for high protein content, selection of high-protein plants in the segregating F_2 generation offers a promising method of attack.

SUMMARY

This study was undertaken to determine, in crosses between Marquis and Kota wheats, the inheritance of yield and crude-protein content, the genetic factors involved for awnedness and dwarfness, and the effect of awns and dwarfs on the yield and protein content of the hybrid plants and strains. The material was grown under favorable conditions in Montana in 1926 and 1927.

The awnleted Marquis parent had outyielded the awned Kota parent in varietal experiments covering the previous five years under favorable conditions at the Bozeman and Moccasin stations but not under unfavorable conditions at the Havre station. The awned Kota parent had exceeded the awnleted Marquis parent in crude-protein content at all three stations.

Dwarf plants appeared in the F_2 generation in a ratio close to 13 normal to 3 dwarf. In the F_3 generation the normal plants bred true or segregated in the ratio of 7:6. The results were explained on a basis of two genetic factors. Dwarf plants caused a significant difference in acre yield of the F_3 rows, but no difference when computed on a basis of yield per plant.

The awnleted and awned classes segregated in the F_2 generation close to a 3:1 ratio. An attempt was made in F_2 to separate the awnleted class into weakly awnleted and strongly awnleted groups. The weakly awnleted plants bred true to the extent of 91.7 per cent, and the strongly awnleted plants segregated to the extent of 87.9 per cent. The F_2 data, corrected on the basis of F_3 results, were close to a 1:2:1 ratio. Both results showed a single genetic factor difference.

Yields of F_2 and F_3 plants averaged intermediate between those of the parent varieties, although in 1926 Marquis was the higher yielding parent and in 1927 Kota was the higher yielding. The yields of the hybrids were more variable than those of the parents. The yields of awnleted F_2 plants and F_3 strains were significantly higher than those of the awned. Greater shattering of awned than awnleted plants and strains was partly responsible for the difference in yield.

The average of the crude-protein content of the F_2 plants and F_3 strains was not significantly different from that of the low-protein Marquis parent. Plants and strains lower in protein content than the extreme of the parent checks but not higher were obtained. There was no difference in average protein content of awnleted and awned F_2 plants, but the awned F_3 strains were significantly greater in protein content than the awnleted, with the segregating strains averaging intermediate.

It is concluded that in crosses between Kota and Marquis wheats grown under favorable conditions at Bozeman, Mont., awnleted selections average higher than awned selections in yield but lower in protein content.

Yield and crude-protein content were negatively correlated in both the F_2 and the F_3 generations, but in neither case was the co-

efficient sufficiently large to be considered important from a plant-breeding standpoint.

The yield of F_2 plants in 1926 and that of F_3 strains in 1927 was positively correlated, but while the coefficient was significant it was hardly large enough to be important.

The crude-protein content of F_2 plants and F_3 strains was correlated positively, and was both significant and important. The results indicate that in breeding for high-protein content the selection of high-protein plants in the segregating F_2 generation offers a promising method of attack.

LITERATURE CITED

(1) BIFFEN, R. H.
1905. MENDEL'S LAWS OF INHERITANCE AND WHEAT BREEDING. Jour. Agr. Sci. [England] 1: [4]–48, illus.

(2) CLARK, J. A.
1925. SEGREGATION AND CORRELATED INHERITANCE IN CROSSES BETWEEN KOTA AND HARD FEDERATION WHEATS FOR RUST AND DROUGHT RESISTANCE. Jour. Agr. Research (1924) 29: 1–47, illus.

(3) ———
1926. BREEDING WHEAT FOR HIGH PROTEIN CONTENT. Jour. Amer. Soc. Agron. 18: 648–661, illus.

(4) ——— FLORELL, V. H., and HOOKER, J. R.
1928. INHERITANCE OF AWNEDNESS, YIELD, AND QUALITY IN CROSSES BETWEEN BOBS, HARD FEDERATION, AND PROPO WHEATS AT DAVIS, CALIFORNIA. U. S. Dept. Agr. Tech. Bul. 39, 40 p., illus.

(5) ——— and HOOKER, J. R.
1926. SEGREGATION AND CORRELATED INHERITANCE IN MARQUIS AND HARD FEDERATION CROSSES, WITH FACTORS FOR YIELD AND QUALITY OF SPRING WHEAT IN MONTANA. U. S. Dept. Agr. Bul. 1403, 71 p., illus.

(6) ——— and SHOLLENBERGER, J. H.
1925. COMPARATIVE VALUE OF KOTA AND MARQUIS WHEATS FOR MILLING AND BREAD MAKING. Northwest. Miller 143: 1102–1104, illus.

(7) GOULDEN, C. H.
1926. A GENETIC AND CYTOLOGICAL STUDY OF DWARFING IN WHEAT AND OATS. Minn. Agr. Expt. Sta. Tech. Bul. 33, 37 p., illus.

(8) HAYES, H. K.
1923. INHERITANCE OF KERNEL AND SPIKE CHARACTERS IN CROSSES BETWEEN VARIETIES OF TRITICUM VULGARE. Minn. Univ. Studies Biol. Sci. 4: [163]–182.

(9) ——— and AAMODT, O. S.
1923. A STUDY OF RUST RESISTANCE IN A CROSS BETWEEN MARQUIS AND KOTA WHEATS. Jour. Agr. Research 24: 997–1012, illus.

(10) WALDRON, L. R.
1926. HYBRID SELECTIONS OF MARQUIS AND KOTA. A COMPARATIVE STUDY WITH REGARD TO DISEASE RESISTANCE, YIELD, AND BAKING QUALITY. N. Dak. Agr. Expt. Sta. Bul. 200, 64 p., illus.

RELATION OF QUANTITY OF SEED SOWN AND DENSITY OF SEEDLINGS TO THE DEVELOPMENT AND SURVIVAL OF FOREST PLANTING STOCK [1]

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INTRODUCTION

It is obvious that seedlings grown in dense stands can not develop so well as those grown without crowding. Nurserymen naturally wish to avoid injury to their stock from crowding, but they also desire to utilize their soil space as fully as possible. The optimum density of stand for each species and age class of nursery stock can be determined within reasonably close limits only by detailed experiments employing plant measurements, weights, root examinations, and observations of survival in the field.[3] Such experiments were conducted at the Savenac nursery in western Montana. Preliminary tests dealt with the effect on seed-bed density of the amount of seed sown and were followed by observation on the evil effects of crowding.

RELATION OF AMOUNT OF SEED SOWN TO DENSITY OF SEEDLINGS

WESTERN YELLOW PINE (PINUS PONDEROSA)

Once the average percentage of viability of the seed is known it is possible, barring irregularities in germination and loss of seedlings, to regulate the density of nursery stands by varying the amount of seed sown. At the Trapper Creek nursery in the spring of 1914 quantities of seed varying from 5 to 20 ounces were sown in drills. The numbers of 1–0 [4] seedlings obtained varied from 600 to 2,226 in direct proportions. Deaths of seedlings from various causes increased in number with the amount of seed sown, but the percentage of loss did not increase with the density of the stand.

The same year at Savenac nursery a similar experiment was made using the broadcast method of sowing. Eight 4 by 12 foot beds were sown in quantities varying from 12.5 to 50 ounces. The nearly constant relation between quantity of seed sown and density of seedlings is shown in Figure 1 by the inclined and almost straight course of curve 1. In these tests also the variations in numbers of seedlings

[1] Received for publication Nov. 2, 1928; issued March, 1929.

[2] Most of the field work on these studies was contributed by E. C. Rogers and P. C. Kitchin, formerly of the Forest Service.

[3] Knowledge of the best density for seedlings is essential, but must be recognized as only a part of the requirements for good seeding practice. In order to obtain the desired density, a nurseryman must also have the results of a germination test of his seed and know how to allow for the difference between germination in greenhouse or laboratory and in the field. Losses from various causes must be anticipated and uniformity in material and methods is essential. The first tests of density of sowing that were made with western white pine failed completely because sowing was done at the wrong time of year and the sand covering for the seeds was not uniform in thickness. Correction of these things in later work led to success of the tests.

[4] In nursery practice the age of plant stock is indicated by figures, the first indicating the number of growing seasons in the seed bed; the second, the number in the transplant bed. Thus 2-0 indicates 2-year-old seedlings; 2-1 indicates 3-year-old stock, 2 years in seed beds, and 1 year in a transplant bed.

lost indicated that although larger numbers of seedlings died, no greater proportion of deaths occurred in the dense than in the less dense beds. Each ounce of seed produced about 260 seedlings.

WESTERN WHITE PINE (PINUS MONTICOLA)

At Savenac nursery in early September, 1919, nine 4 by 12 foot beds were sown broadcast with lots of seed varying from 222.5 to 890 gm. Two years later estimates were made of the number of 2-year-old

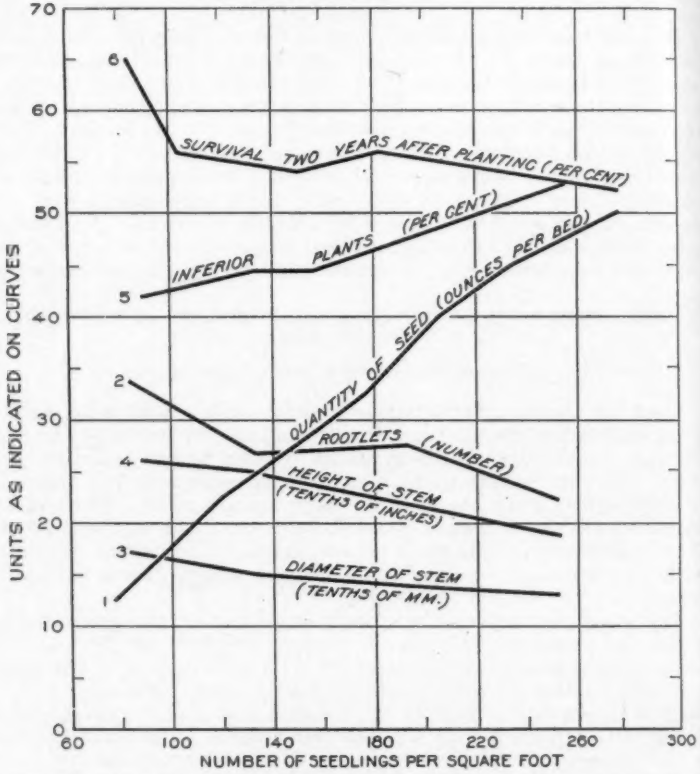

FIG. 1.—Effects of density of seedlings on the development and survival of 2-0 western yellow pine stock when various quantities of seed are sown

seedlings by counting them on strips covering one-quarter of the area. The number of seedlings per square foot varied approximately from 160 to 480 according to the amount of seed sown. Curve 1 in Figure 2 shows a nearly constant relation between the amount of seed sown and the density of the seedling stand.

ENGELMANN SPRUCE (PICEA ENGELMANNI)

Six identical 4 by 12 foot beds were sown at Savenac nursery in the spring of 1915, each with a different quantity of seed. These beds

were prepared in the ordinary manner, sown broadcast, covered with sand one-quarter inch deep, protected by screen frames, shaded alike, and watered quite uniformly. Throughout the summer frequent counts were made of the numbers of seedlings lost from different causes on sample plots in each bed. These observations revealed a fairly constant relation between amount of damping off and density. In September all living plants in these beds were counted, with the results shown in Table 1. The stands corresponded quite closely to the quantities of seed sown, demonstrating that it is possible to control density within fairly close limits by sowing more or less seed.

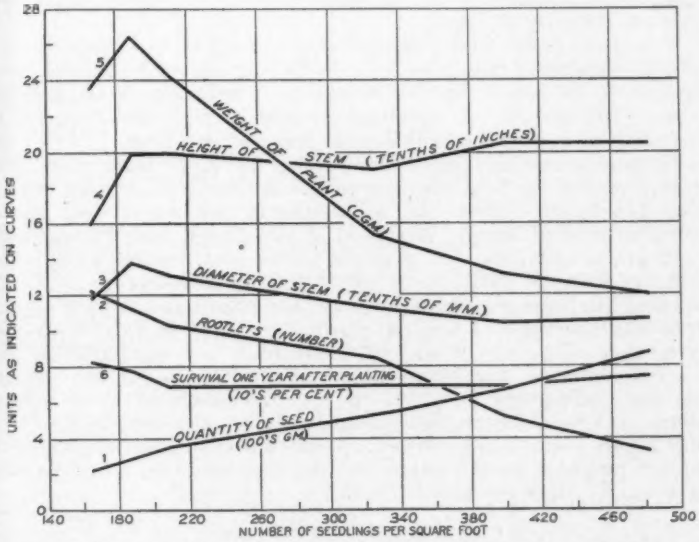

FIG. 2.—Effects of density of seedlings on the development and survival of 2-0 western white pine stock when various quantities of seed are sown. The quantity of seed is shown in hundreds of grams, that is, 4 on the scale equals 400 gm.; survival is indicated in tens per cent, that is, 8 on the scale equals 80 per cent

TABLE 1.—*Density of 1–0 Engelmann spruce seedlings as related to the amounts of seed sown*

[Figures are based on two seed beds of 48 square feet representing each density class]

Amount of seed sown per bed (48 square feet)	Average Density at end of first season by survival count		Theoretical density calculated at a rate of 200 plants per gram of seed
	Per bed (48 square feet)	Per square foot	
Grams	*Number*	*Number*	*Number*
27.18	5,740	120	5,436
36.25	7,177	150	7,250
45.31	9,370	195	9,062
54.37	10,305	215	10,874
63.44	11,100	231	12,688
72.50	13,439	280	14,500

RELATION OF DENSITY OF SEEDLINGS TO ANATOMICAL DEVELOPMENT

WESTERN YELLOW PINE

From each of the eight beds of 1–0 western yellow pine at Savenac, which varied in density from about 80 to 275 seedlings per square foot, 100 plants were removed and measured. Averages from the figures so obtained revealed no significant differences in height of top, but they indicated a gradual decrease in diameter of stem with the increase in density. Curve 3 in Figure 1 marks an average decrease of 0.1 mm. in diameter for each increase of about 44 trees per square foot. The number of lateral roots also diminished steadily with increased density of stand.

Two years later similar results were obtained from measurements of 3–0 stock from the same beds. The tendency for the number of rootlets to be less in the denser stands is apparent in the trend of curve 2 in Figure 1. It indicates an average decrease of one rootlet for each increase of about 14 seedlings per square foot.

Numerous inferior plants were found in these beds when trees were removed for field-planting tests. Although they were 2 years old, these plants either had no needles in fascicles or only two or three bundles of them. The stem of the second year's growth was light green and bore flat straplike leaves of the same color, quite different from the dark green bushy top of a normal seedling. Terminal buds on these plants did not have the brown and mature appearance characteristic of normal plants. In another experiment this type of seedling was found to result from late germination,[5] but there is evidence that plants weakened in other ways may develop the same abnormal form. Thus the percentage of these inferior plants was found to increase with density in the seed bed. Curve 5 in Figure 1 shows this relation. It indicates an increase in distinctly inferior plants equal to 1 per cent of the total stand for every increase of about 15 trees per square foot.

WESTERN WHITE PINE

From the nine beds originally sown with different quantities of seed, two hundred 2–0 seedlings were taken from each of six stands of different density. Averages of weights and measurements of these trees, although not entirely consistent throughout, clearly showed some of the effects of crowding. Although the trees grown in dense stands were slightly taller (fig. 2, curve 4) than those having more room, they were decidely more slender (curve 3), and were of inferior development, as indicated by plant weights (curve 5). The number of lateral roots within 8 inches of the surface decreased quite rapidly with increases in density. (Curve 2.) When trees were lifted from the densest lot it was noticed that their roots had penetrated much more deeply than the others. Such growth is undesirable because roots more than 8 inches long can not be properly planted in the field by present methods.

[5] WAHLENBERG, W. G. SOWING AND PLANTING SEASON FOR WESTERN YELLOW PINE. Jour. Agr. Research, 30: 245–251. 1925.

ENGELMANN SPRUCE

In the spring of 1917 about 200 of the 2–0 spruce seedlings grown at each of six different densities were washed out of the beds at Savenac nursery. While stored in a root cellar awaiting laboratory examination the tops of the trees mildewed so badly that no measurements of them could be made, but measurements of the roots are given in Table 2. The decrease in number of rootlets and in diameter of stem with increased density is clearly shown. In September of the same year, when the plants remaining in the nursery beds were 3–0, about 400 were removed by washing from each of six beds of different densities. The average results from measuring both roots and tops of these plants are given in Table 3. With one exception, all the characteristics desired of planting stock seem to decrease with the increase of density in the seed-bed stand. The trees from dense stands are better balanced than those from thin stands, that is, they have a larger portion of their total weight in roots. This is known to be an advantage, but in this case it was gained through puniness of tops rather than through good development of roots, and was completely outweighed by the advantage of better general development attained in the thinner stands.

TABLE 2.—*Measurements, weights, and counts of 2–0 Engelmann spruce roots on seedlings grown at different densities*

[Basis: 200 plants from each different density. Primary lateral rootlets are branches from the central or taproot. Secondary laterals are branches from the primary rootlets.]

Density of seedlings per square foot of seed bed	Diameter of stem at ground line	Primary lateral rootlets		Secondary lateral rootlets		Weight of fresh, washed, air-dried roots
		Diameter 0.5–2 inches	Diameter over 2 inches	Diameter 0.5–2 inches	Diameter over 2 inches	
Number	*Millimeters*	*Number*	*Number*	*Number*	*Number*	*Grams*
116	1.36	20.5	7.0	5.3	0.1	0.08
138	1.29	18.3	5.8	5.0	.1	.06
184	1.24	19.4	6.0	4.6	.1	.06
191	1.23	17.5	6.3	4.6	.1	.06
217	1.14	17.7	4.9	3.0	.0	.05
323	1.12	15.9	4.3	1.8	.0	.05

TABLE 3.—*Measurements and weights of 3–0 Engelmann spruce seedlings grown at different densities*

Density of seedlings per square foot of seed bed	Length of stem [a]	Diameter of stem [b]	Length of needle [c]	Weight air dried [d]			Ratio roots to plant	Plants examined
				Top	Roots	Total		
Number	*Inches*	*Mm.*	*Inches*	*Gm.*	*Gm.*	*Gm.*	*Per cent*	*Number*
116	1.58	1.53	0.48	0.38	0.12	0.50	24.0	428
161	1.37	1.40	.44	.27	.09	.36	25.0	395
206	1.24	1.20	.41	.18	.08	.26	30.8	387
191	1.35	1.31	.40	.18	.08	.26	30.8	399
217	1.17	1.14	.38	.11	.06	.17	35.3	394
323	1.10	1.03	.36	.10	.06	.16	37.5	380

[a] Ground line to tip of terminal bud.
[b] At ground line.
[c] Needle taken from middle of crown.
[d] Fresh washed plants.

RELATION OF DENSITY OF SEEDLINGS TO FROST RESISTANCE

No study was made of frost action on western yellow pine and western white pine because frost does not ordinarily interfere seriously with the growth and survival of these species.

ENGELMANN SPRUCE

Seedlings may be injured by frost in two ways. Frost injures the tops by freezing and killing the tissue. Frost heaving injures the roots by breakage or exposure, the roots being lifted from the soil by the expansion of ice crystals. Spruce seedlings are subject to both of these forms of frost injury in western Montana.

In May, 1918, at Savenac nursery the relation between density of spruce seedlings and frost nipping was investigated on 25 sample plots of 1 square foot each representing average conditions in stands of different density. The results of this examination, as given in Figure 3, indicate a decided advantage in density of stand, for while

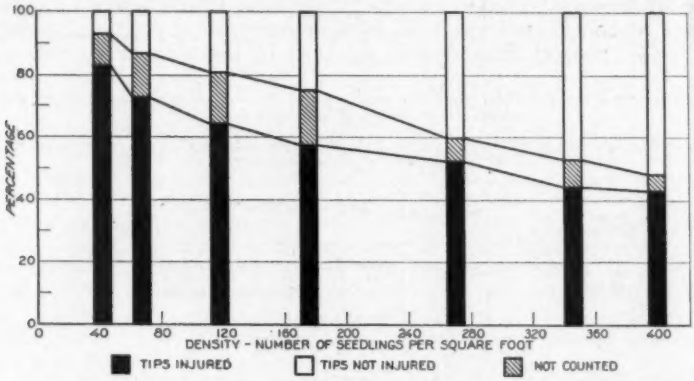

FIG. 3.—Relation of density in seed bed to frosting of tips of Engelmann spruce seedlings

a stand of but 42 seedlings to the square foot had only 6.5 per cent of the tips uninjured, that with 400 seedlings escaped with nearly 52 per cent of the tips untouched by frost.

This single series of observations indicates that the susceptibility of Engelmann spruce seedlings to frost nipping depends upon density in the seed bed, and that the greater the density the less is the injury by frost. This may be due to a layer of comparatively warm air, held in place by a thick mat of plants, tending to equalize air temperature at the surface of the bed and to prevent the freezing temperatures that would occur in more open stands.

More severe damage was caused by frost heaving. This was also studied in 1918, in transplant beds and in field plantations set out the previous year. One thousand 2-0 seedlings were transplanted from each of the beds of different density (as shown in Table 1) early in June, 1917, and in October eight hundred 3-0 seedlings from each bed were planted in the field. The field planting was divided equally between two widely separated areas. When examined in the spring of 1918 the trees were classified as follows;

Class 1. Plant erect, thrifty, and with roots firmly embedded in the soil.

Class 2. Plant heaved by frost, perhaps prostrate, but with some roots firm enough in the soil to withstand a pull of about 2 ounces; having a chance to live, but handicapped.

Class 3. Plant heaved by frost, apparently dead; unable to withstand a pull of about 2 ounces or thrown completely out of the soil; most of the root tips showing no life or having no contact with the soil.

The result of using this classification of transplants is given in Table 4. These figures seem to show that frost heaving of 2-1 Engelmann spruce in transplant rows increases with the seed-bed density at which the trees were grown, especially for densities greater than 150 per square foot. Similarly this same tendency was shown to hold true for the 3-0 stock in both field plantations, one on a northwest exposure near Wallace, Idaho, and the other on a southwest exposure near Haugan, Mont.

TABLE 4.—*Degree of injury by frost heaving sustained by 2-1 Engelmann spruce transplanted from seed-bed stands of different densities*

Density of seedlings per square foot of seed bed	Uninjured	Frost heaved	Fatally frost heaved	Plants examined
Number	*Per cent*	*Per cent*	*Per cent*	*Number*
120	33.6	24.0	42.4	921
150	31.7	31.5	36.8	904
195	12.0	21.3	66.7	841
215	2.9	16.0	81.1	784
231	4.6	21.4	74.0	778
280	2.6	10.6	86.8	774

RELATION OF DENSITY OF SEEDLINGS TO SURVIVAL IN THE FIELD

WESTERN YELLOW PINE

That more of the normal than of the inferior plants survived planting was to be expected. The difference in their behavior is shown in Table 5. Although they constituted but 48 per cent of the aggregate stock, the inferior class of plants furnished 57 per cent of the unthrifty and 59 per cent of the dead plants at the end of the first season in the field. To a large extent the low survival of trees from the dense seed-bed stands (fig. 1, curve 6) was attributed to the presence of numerous inferior plants of the type described. The curve indicates that after two years in the field the percentage of the total number surviving planting decreased about 1 per cent for each increase of 15 trees per square foot in the original seed-bed stands.

TABLE 5.—*Relative survival of 2-0 western yellow pines of the normal and inferior types planted in the spring of 1916*

Date of examination and type	Thrifty	Unthrifty	Living	Dead
July, 1916:	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Normal	67.7	24.0	91.7	8.3
Inferior	60.3	21.1	81.4	18.6
November, 1916:				
Normal	70.0	13.6	83.6	16.4
Inferior	57.9	18.1	76.0	24.0

WESTERN WHITE PINE

Three hundred plants representing each of six densities were planted on a north slope in the fall of 1921. After one year, survival was somewhat higher for densities of less than 210 seedlings per square foot. (Fig. 2, curve 6.)

ENGELMANN SPRUCE

Four hundred seedlings from each of six different densities of 3–0 stock were planted on a southwest slope and a duplicate plantation was set out on a northwest slope in the fall of 1917. The figures obtained in observing survival during the next three years were neither striking nor entirely consistent, but they indicated the tendency toward higher survival of the trees grown at the lesser densities.

RELATION OF DENSITY OF SEEDLINGS TO GROWTH IN THE TRANSPLANT BED AND IN THE FIELD

WESTERN YELLOW PINE

In the spring of 1915 about one thousand five hundred 1 year-old western yellow pine seedlings from each of the eight seed beds of different density were transplanted. In the fall of 1916 the growth in height during the year and the total height of the plants were measured on 200 trees in each of the different lots. Curve 4 in Figure 1 shows the decrease in height growth of the transplants with the increase in their density as seedlings and with the increase in number of inferior plants. Average height growth decreased about 0.1 inch for each increase of 24 seedlings per square foot of seed bed.

Similar figures were obtained from measurements of height growth of 2–0 yellow pine stock two years after field planting in the spring of 1916.

No growth measurements were made of western white pine.

ENGELMANN SPRUCE

Height growth during the second and third seasons in the field was measured on 100 or more trees from each of the various lots grown at different densities in the seed bed. The growth averages obtained, like those of survival, were neither striking nor entirely consistent, but indicated the tendency toward faster growth of the trees grown at the lower densities.

CONCLUSIONS

Density of 1-year-old western yellow pine seedlings varied directly as the amount of seed sown per square foot. Working with seed-bed densities varying approximately from 80 to 275 seedlings per square foot, the following average results were attained.

The stem diameter of 1–0 seedlings decreased 0.1 mm. for each increase of about 44 trees per square foot. The height growth of 1–2 transplants in the nursery decreased one-tenth inch for each increase of about 24 seedlings per square foot of seed bed. The number of rootlets on 3–0 stock decreased by one for each increase of about 14 seedlings per square foot. The percentage of distinctly inferior 2-year-old plants increased by one for every increase of about 15 trees per square foot. These differences in anatomical

development, as a result of differences in seed-bed density, had their effect on the survival of 2–0 stock in the field. Two years after planting, survival was observed to be 1 per cent lower for each increase of about 15 trees per square foot of the original seed bed. Height growth in the field also decreased with increases in density of the seed beds.

Working with densities varying approximately from 160 to 480 seedlings per square foot, the following results were attained with western white pine:

Two-year-old trees grown in dense stands were much smaller, more slender, and slightly taller than those having more room. The crowded trees also possessed fewer lateral rootlets. Field survival was distinctly higher for trees grown at densities of less than 210 seedlings per square foot.

With 3–0 Engelmann spruce ranging in density from 116 to 323 seedlings per square foot, increases in density were accompanied by decreases in characteristics desirable for planting stock. Decreases in length and diameter of stems and in length of needles were observed as well as decreases in the weight of the tops and roots. Such decreases in general development overshadowed the advantage from an increase in the portion of total plant weight contained in roots of densely grown plants. Frost nipping in spruce seed beds at Savenac nursery decreased with increases in density, but the more severe damage from frost heaving acted differently. Frost heaving of 2–1 spruce in transplant rows and 3–0 spruce in field plantations increased with the density at which the trees were grown in seed beds. The field plantations showed a tendency toward increased survival and growth with decreases in the density of original seed beds.

For the best development of seedlings at Savenac nursery, there should be less than eighty 2-year-old western yellow pines, less than one hundred and sixty 2-year-old western white pines, and less than one hundred and twenty 3-year-old Engelmann spruce per square foot of seed bed surface.

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EXPERIMENTS IN THE SILVICULTURAL CONTROL OF NATURAL REPRODUCTION OF LODGEPOLE PINE IN THE CENTRAL ROCKY MOUNTAINS [1]

By CARLOS G. BATES, *Silviculturist*, and HUBER C. HILTON and THEODORE KRUEGER, *Forest Supervisors, Forest Service, United States Department of Agriculture*

INTRODUCTION

The natural regeneration of lodgepole pine (*Pinus contorta*) has long been a matter of great interest because this species is very prolific, leading to stands of seedlings, saplings, and poles of extraordinary density, and hence giving rise to the slender, unbranched stems which make possible the use of the tree wherever a clean, untapering pole is desired.[2]

The earliest observers (for example Clements,[3] Mason,[4] and others) have noted that the typically dense stands of lodgepole usually followed fires that completely or almost completely destroyed the parent trees, leaving the ground free of any spongy covering and as devoid of any weed-growth for a number of years as it had been under the old forest. On such a seed-bed the lodgepole seedlings, completely lacking in competition for moisture or light, throve because they were the first comers. Seeds, retained year after year in the tightly closed cones on the branches of old trees, were possibly released within a few days after the fire, even though this had "crowned" and burned off the foliage in a flash of flame. Also, a fire which does not reach into the tops of the tallest trees, or which does so only in spots, may readily "roast" every vestige of foliage, killing the trees outright, and yet furnish about enough heat to guarantee the opening of any cones which may be clinging to the trees. Earlier experiments by Bates have shown that, on the average, seeds so released are likely to comprise the equivalent of three normal yearly seed crops.

But forest management does not recognize fire as a legitimate silvicultural agent, at least in the Rocky Mountain region where every particle of organic matter is needed to build up poor, thin soils. If fire is used at all, it must be after cutting, to remove at a propitious time that coarse but unmerchantable material which, for a few years, would make an accidental fire, once started, difficult to control. But even slash burning, because of the injury which results so often to standing trees, is rapidly being abandoned except in spots or lanes where it is especially needful to remove all risk of fire starting.

The early plans for forest management may, then, be said to have encountered two fairly distinct problems, which the studies recounted in this article and other observations of a less systematic nature,

[1] Received for publication Nov. 2, 1928; issued March, 1929.

[2] In somewhat open stands, in which it is sometimes found, lodgepole develops a form of crown and bole little different from that of the other pines.

[3] CLEMENTS, F. E. THE LIFE HISTORY OF LODGEPOLE BURN FORESTS. U. S. Dept. Agr., Forest Serv. Bul. 79. 56 p., illus. 1910.

[4] MASON, D. T. THE LIFE HISTORY OF LODGEPOLE PINE IN THE ROCKY MOUNTAINS. U. S. Dept. Agr., Bul. 154. 35 p., illus. 1915.

have assisted in solving. These may be stated in the form of questions.

1. Is fire necessary, even to the extent of burning the needle litter, to insure adequate reproduction of lodgepole stands which are cut clear or opened up by thinning?

2. Can the very dense stands which seem to be the usual order resulting from the burning and "spontaneous" regeneration of lodgepole, and which lead to slow growth or complete stagnation while the trees are yet small, be prevented and controlled in managed forests so that young stands will develop to commercial size within a reasonable rotation, and without the expense of early artificial thinnings?

By anticipating somewhat the results to be described later, these questions may be answered by saying that it is now quite evident that fire is not a necessary agent for lodgepole regeneration; that the most that it does is to clear a space for seedlings and to release a great mass of seeds at the earliest moment, which may or may not be an opportune time for their germination, as determined by subsequent weather conditions; and that cutting, to the degree that it makes space for seedlings, is almost as effective as burning, the seed necessary for an adequate crop of young trees being obtained either from the trees left—in partial cutting—from trees on the side, or from slash left on the ground in clear-cutting. Moreover, as neither the gradual release of seed following cutting, nor the forest floor conditions where the slash is left unburned, are conducive to "spontaneous" germination, but rather to a gradual appearance of seedlings, the danger of overstocking is largely removed. Excessive density is not likely to occur, or at least to be maintained for any length of time, except where the competitors are almost identical in age, size, and vigor. In other words, the disposal of slash without burning is a better guarantee of uniform stands of reproduction, avoiding both extremes.

DESCRIPTION OF THE EXPERIMENTS

Two experiments to determine the conditions conducive to reproduction of lodgepole pine were started in 1909 and 1912 respectively. The first was on the Medicine Bow National Forest on the south boundary of Wyoming; the second on the Gunnison National Forest a little southwest of the center of Colorado. The physical conditions and character of stands in the two localities are quite different, yet the reproduction results are qualitatively very similar.

The Medicine Bow area is a flat bench at 9,000 feet elevation, with a soil derived from gneiss. The soil and climatic conditions are conducive to fairly rapid growth of lodgepole in the aggregate, although the stand treated averaged only about 9 inches d. b. h.[5] at 200 years, owing to its great density. The conditions are also conducive to heavy reproduction, as attested by excessively dense stands of various ages in the immediate vicinity.

Under these conditions eight plots (fig. 1) of about 0.59 acre each were laid out, two to a strip on four strips running north and south. Strip II was clear cut, Strip III was given a thinning distributed as

[5] D. b. h. = Diameter at breast height.

evenly as possible, and Strip IV was thinned in groups designed to leave openings from 20 to 40 feet in diameter.[6]

The thinnings removed from each of the plots on Strips III and IV equalled 30 to 35 per cent of the original volume, any small differences being insignificant in comparison with the variations in distribution of the cuts. On Strips II, III, and IV the brush was disposed by burning on one plot and by scattering on the other. Nothing, however, was done to either of the check plots on Strip I. Each square plot was divided into 100 equal sections 16 feet square, on each of which the reproduction was recorded separately.

Strip I lay between the clear-cut strip on its west and a similar cleared right-of-way, 200 feet wide, on its east. It is unfortunate that this "check" or uncut area adjoined the clearing on both sides, without any isolation strip, since the exposure on the west resulted in the windfall of several trees after the experiment was started, and this, of course, made openings for reproduction. In fact, the lack of

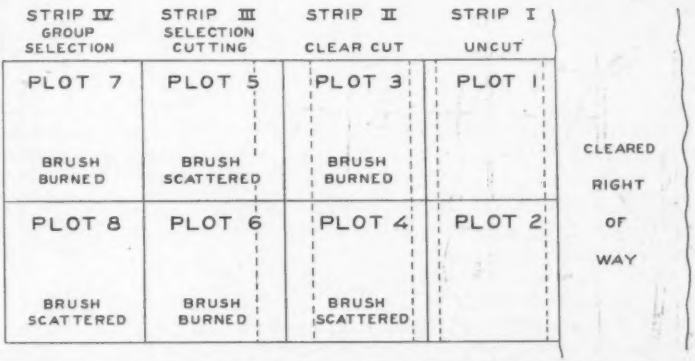

FIG. 1.—Arrangement and treatment of eight experimental plots at Medicine Bow National Forest, 1919. Each plot was a square, 160 feet on each side, and was divided into one hundred 16-foot square sections. Dotted lines indicate areas eliminated

isolation strips makes it necessary to disregard the edges of the plots on this and other strips in order to obtain normal conditions.

Measurements made at 50 points on the four thinned plots, with the Clements's photometer, indicated about 39 per cent of full sunlight received on the average, although one would readily say, looking directly upward at the crowns of an undisturbed lodge-pole stand, that not 50 per cent of the space was occupied. Because of the openings on both sides, the plots on Strip I received only a little less light, or 35 per cent, while those on Strip II, with walls of trees on the east and west, received 76 per cent of the full sunlight of a summer's day. But probably within these limits the amount of light admitted by cutting is much less important in controlling reproduction than is the root space freed, which may be considered roughly proportionate.

The Gunnison experiment followed an entirely different plan and was somewhat less intensive. The country rock is granite and the

[6] It should be pointed out that the occurrence in the last named areas of a few trees about 60 years older than the main stand—such trees usually surrounded by greater or less open space—made the areas peculiarly suitable for this method of cutting.

soil is slightly gravelly, with sufficient clay to give it body. It appears to be a less productive soil than that of the Wyoming area. The timber is smaller and less vigorous in appearance. On a steep slope facing almost to the northwest (except at the southwest end, where it rounded to a more westerly aspect) seven long plots extending up

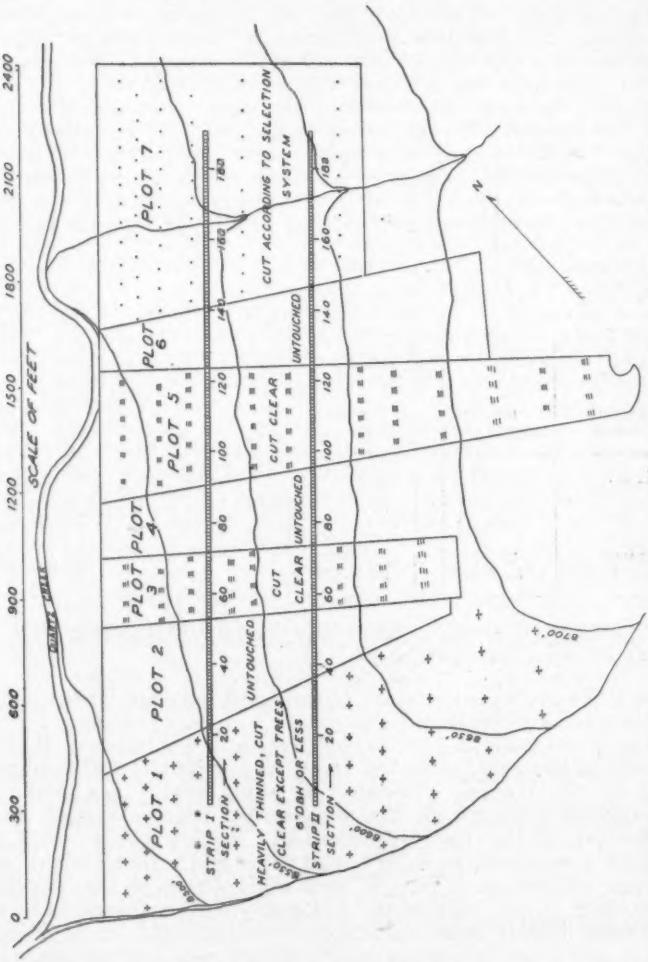

FIG. 2.—Arrangement of seven irregularly shaped experimental plots on the Gunnison area. The two 10-foot wide observation strips are parallel at a distance of about 300 feet and are divided into one hundred and ninety 10-foot sections. No plot at point of transect is less than 170 feet wide

and down the slope a distance of about 1,000 feet were laid out. (Fig. 2.) The aggregate width of these plots was slightly more than 2,400 feet. Individually they were of variable widths and no two were of like dimensions, but at no point where the measurements were made was any plot less than 170 feet in width.

Of the seven plots, 2, 4, and 6 were left in their original condition. Plot 1, on the southwest end, was very heavily thinned, leaving only small, suppressed poles under 6 inches d. b. h., which later observation has shown to possess little power of recovery. Plots 3 and 5 were clear-cut. Plot 7 was cut about in accordance with the practice followed on national forest timber sales of that time, a fairly light selection or improvement cutting—but relatively a somewhat heavier cutting than that on Strip III of the Medicine Bow series. Table 1 gives some comparative stand data from samples of the Gunnison plots and from the Medicine Bow plots as a whole.

TABLE 1.—*Comparison of stands left in the plots on the Gunnison and Medicine Bow National Forests as shown by 1924 record*

Forest and description of cutting	Trees per acre	Total basal area	Average d. b. h.
	Number	*Square feet*	*Inches*
Medicine Bow:			
Virgin stand	608	[a] 205.90	7.88
Even selection cutting	475	196.64	8.71
Group selection cutting	450	169.63	8.31
Gunnison:			
Virgin stand	630	147.27	6.55
Selection cutting	275	79.99	7.30
Heavy cut [b]	810	55.98	3.56

[a] About 218 square feet at beginning of experiment. Windfall losses were largely balanced by growth up to 1924.

[b] This refers to the heaviest portion of the stand covered by sections 21–36 of Strip II. Elsewhere the average stand is estimated at 50 such trees per acre.

Across the seven Gunnison plots two transects 10 feet wide were laid out in 1913, on which reproduction was counted in that year and again in 1916, 1919, and 1924. The first of these strips was about 300 feet from the foot of the hill, and approximately at right angles to the slightly irregular plot lines, the second was 300 feet farther up the slope and parallel to the first. Each strip was divided into one hundred and ninety 10-foot sections, on each of which reproduction and cover conditions were recorded separately. Although sections of corresponding number in the two strips are exactly opposite, the strips do not intersect the plot lines at the same distances from the points of origin. Thus section 92 of Strip I falls on the east side of a dividing line and in a clear-cut area, whereas the same number in Strip II is on the west side of the line and in an uncut stand. For this reason the records of the two strips can not be averaged section by section but must be considered on their separate merits.

Although the slash was burned on a few small spots within these areas, the reproduction strips do not touch any such areas. On the other hand, the dragging of logs in skidways down the steep slope through the clear-cut areas left certain lanes stirred up and somewhat devoid of slash, while at the sides of these the loppings were more or less bunched. The locations of these lanes are indicated later.

RESULTS

MEDICINE BOW AREA

Partial counts on the eight plots of the Medicine Bow area were made in 1912–13, and 1915, and a final, more complete count in 1919, when all of plots 3, 4, 6, 7, and 8 were covered, and 20 per cent of

plots 1, 2, and 5, where it was evident there were no wide variations. Although discussion in detail will be confined to this last count, it is well to show the progress of reproduction by giving the totals for the intermediate examinations, as in Table 2.

TABLE 2.—*Gross figures showing progress of reproduction on the Medicine Bow areas after cutting in 1909*

Strip and treatment	Plot No.	Approximate number of seedlings on the plots in—		
		1912–13*	1915	1919
Strip I:				
Uncut	1	6,210	8,700	17,565
Do	2	5,970	7,700	13,930
Strip II:				
Clear-cut, brush burned	3	3,135	7,750	9,288
Clear-cut, brush scattered	4	2,710	3,755	4,285
Strip III:				
Even selection, brush scattered	5	6,750	10,650	15,600
Even selection, brush burned	6	8,145	20,750	41,090
Strip IV:				
Group selection, brush burned	7	12,012	22,871	36,755
Group selection, brush scattered	8	4,996	7,431	12,911
Total		49,928	89,677	151,424

* First four plots counted late in 1912, others in June, 1913. Of the total number at this time, 3,681, or about 7 per cent, were classed as more than 3 years old, but if seedlings to this number were on the ground at time of cutting, they must have been of a very inconspicuous class, as described later.

These gross figures indicate a steady increase in the number of seedlings on the ground, the number present three years after the cutting being nearly doubled in an additional three seasons, and again in the last 4-year period. However, too much significance should not be attached to these figures, since it was true in 1919, and presumably also in 1915, that the majority of the seedlings were too small and poorly established to be counted as practical assets unless liberated by further cuttings. On the other hand, the very large numbers in some plots might well become a menace if given full opportunity to develop.

Especially noteworthy is the decreasing number of additional seedlings on the clear-cut area, which has always had much fewer seedlings than even the virgin stand. Despite this lag in numbers the clear-cut area has the advantage in that the seedlings have been actually developing from the outset.

In all three pairs of plots it is evident that brush burning is an advantage to seedlings, but a disadvantage to silviculture in that it may easily make possible too much reproduction.

Attention may now be turned to a more critical interpretation of the results recorded in 1919. Without attempting to determine the age of seedlings, all were at this time recorded in inch height classes. The seedlings recorded as 1 inch in height, and less, are of special interest because most numerous. This group includes only a few seedlings of 1919 germination, and many at least 3 years old. The average age of the group is estimated, from the appearance of the seedlings, to be about 2 years. Under the conditions of the virgin forest, and to a lesser extent in the thinned stands, it is believed that a con-

siderable crop of seedlings starts every year—possibly as many as ten to fifteen thousand per acre in the average year. Most of these probably live about three years without having made appreciable growth beyond three or four tufts of needles. They then succumb, not regularly, but in the drier periods, either because they have not had sufficient light to make vigorous root growth, or because, if the roots do penetrate into the mineral soil, they find it occupied and drained by the well-established roots of older trees. Those that remain are still in considerable numbers, and that many of them become established, or at least persist for long periods, is indicated by the steady increase in total numbers on the plots. However, in an attempt to measure the successful reproduction at the end of 10 years in this experiment, this smallest class, at least, must be excluded, and conclusions must be based on the numbers which have found opportunity for appreciable growth.

Before attempting to compare the more typical interior portions of the several plots it is desirable to note the distribution from east to west as influenced in each pair of plots by the character of the cutting adjacent to its side lines.

For this purpose each plot may be considered to be divided into 10 tiers of 10 sections each, numbered 1 to 10 from east to west. In plots 1 and 2 the extreme east and west tiers (1 and 10), because of being well lighted from the sides and also because of windfalls along the edges, contained a few well-developed seedlings which were not typical of the interior of the plots, and were therefore omitted. (See broken lines in fig. 1.)

In plots 3 and 4, the tier along the east edge appeared to be especially favored for germination and could well be eliminated because it was shaded nearly half the day. Although the number of seedlings of the larger sizes was not at all unusual, this may possibly have been due to the roots which penetrated this strip from the trees of plots 1 and 2. Somewhat the same situation might have been expected to exist along the west side of plots 3 and 4, but here the number and size of the trees suggested rather that the conditions that were not typical of what would occur on a more extensive clearing may have been due to snow drifting. In other words, this edge received an excess of water, and for this reason the two westernmost tiers were excluded.

In plots 5, 6, 7, and 8 the number and size of seedlings were so largely influenced by the small or large openings, and especially by the spots on which brush was burned, that the distribution was very irregular as recorded in the arbitrary 16-foot sections. It was, however, quite evident that the two easternmost tiers of sections of 5 and 6 were influenced by the opening on that side and could therefore be omitted as not typical. On the west boundary of this strip, and on both sides of plots 7 and 8, the conditions are practically the same as in the interiors of the plots and no eliminations are necessary.

Table 3 gives the number of seedlings per acre based on the counts for the interior portions of the plots not eliminated above.

TABLE 3.—*Number of seedlings per acre for the typical interior area of each experimental plot, Medicine Bow Forest, 1919*

Plot No.	Sections counted	Seedlings per acre, by specified diameter groups					Totals			Average height of trees over 1 inch in diameter [a]
		New and 1 inch	2–5 inches	6–11 inches	12–17 inches	18 inches +	All sizes	2 inches and over		
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*		*Per cent*	*Inches*
1	[b] 16	26,848	1,428	261	34	0	28,571	1,723	6.0	4.4
2	[b] 16	24,252	1,667	510	136	11	26,576	2,324	8.7	4.9
3	[c] 70	5,204	3,124	3,002	1,311	590	13,231	8,027	60.7	8.2
4	[c] 70	731	1,005	1,231	660	493	4,120	3,389	82.3	10.5
5	[d] 16	22,979	2,213	202	32	0	25,426	2,447	9.6	5.0
6	[d] 80	63,498	7,009	1,591	334	126	72,558	9,060	12.5	5.1
7	100	55,002	5,243	1,526	451	287	62,509	7,507	12.0	5.2
8	100	18,100	2,435	906	316	199	21,956	3,856	17.6	6.0

[a] Excluding the large number of unestablished new and 1-inch seedlings.
[b] Eliminating east and west tiers of sections because of clear-cutting on both sides of plots; using 0.441 acre as partial area. A 20 per cent count was made on these plots.
[c] Eliminating east tier on account of shade, two west tiers on account of shade and snow-drifting; using 0.412 acre as partial area.
[d] Eliminating two east tiers on account of light from clear-cut area; using 0.470 acre as partial area. A 20 per cent count was made on plot 5.

Only those seedlings which have exceeded in size the 1-inch class described above are considered as "growing" and as real reproduction assets; the real difference between the clear-cut plots and all of the others may readily be seen by reference to the next to last column in Table 3, where it is seen that on the typical areas only 6 to 18 per cent of the seedlings are developing under the cover of older trees, while in the clearing 60 to 80 per cent are progressing. And here the remaining trees are not necessarily old or suppressed, but may well be new seedlings still coming in.

The large number of seedlings 1 inch and smaller, 25,000 to the acre, which exists in the uncut stand, many of which would be ready to spring into active growth if the overstory were removed, is a strong commentary on the reproductive capacity of this species, and upon the favorable conditions for natural reproduction which seem to prevail in most of the higher portions of the central Rocky Mountains. There are in this stand, in fact, enough of the seedlings making appreciable growth to guarantee the future of the forest, if all might escape damage during the cutting. Their distribution, however, is very irregular.

Only on plots 6 and 7 where the brush was burned, have the partial cuttings increased appreciably the total numbers of seedlings over those of the virgin stands, but in all the partially cut plots somewhat larger proportions of the seedlings are developing. The number of small seedlings is increased throughout the burned plots, and the larger ones in the openings are very noticeable. The burning of the slash in piles accomplished considerably more than preparing a "favorable seed bed." Besides killing some trees around the spots, and weakening others, it evidently killed the more superficial roots which extended into such areas from the trees left around them, and this made available moisture which permitted the seedlings starting in these areas to grow. Although they are by no means like the better seedlings of the clear-cut area, they are vigorous in compari-

son with all others in these same stands, and form definite clumps, as illustrated in Figure 3. The average height, particularly in plot 6, is held down by the enormous number of small seedlings.

Although it is difficult to picture the situation exactly from the records made by sections, it is believed from ocular observations that in plot 5, where only small openings were made and the brush was scattered, the cutting has had practically no influence on the number or distribution of seedlings, and only slight influence on the size. In plot 8, with its larger openings, the seedlings are quite well developed in the openings, and there is also a fairly large number of small ones in the intermediate spaces.

F-230150

FIG. 3.—Clump of reproduction on burned spot on plot 6, Medicine Bow National Forest, July, 1919. Note the contrast with the remainder of the area, where there was some cutting, but no burning

Notestein made a very exhaustive study of the physical conditions of these plots in 1912, and his conclusions exploded the idea that the spots of ground where the humus was destroyed by fire were particularly favorable for immediate germination by reason of more effective surface conditions. He found relatively few seedlings in the centers, but many around the edges of these spots where cones, as well as coarser material of the piles, had escaped burning. Later, it is true, a few seedlings started, and developed rapidly, in the centers of the spots. This is in agreement with the findings of Mason.[7]

It is, then, quite evident that even a comparatively light cutting in a mature lodgepole stand makes space for reproduction, and that, at least under the conditions here described, lack of reproduction is

[7] Mason, D. T. UTILIZATION AND MANAGEMENT OF LODGEPOLE PINE IN THE ROCKY MOUNTAINS. U. S. Dept. of Agr. Bul. 234. 54 p., illus. 1915.

not likely to give concern, since much is already established, if feebly, before any cutting has taken place. Where twice as much volume is removed as from these plots—a cutting practice in accord with that on national forest timber-sale areas—there is usually a good deal of reproduction established within a few years over most of the area, even though trees left in clumps make it decidedly patchy. It remains to be seen whether, by removing the original stand in two cuts, too much reproduction will in general be obtained.

Advantages of Clear-Cutting and Scattering Slash as Indicated by the Medicine Bow Plots

Interest must center on the results attained by clear-cutting, even though this practice may not be universally feasible because of the nature of the stands, the limited market for small poles, and the public

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Fig. 4.—Evenly distributed reproduction on plot 3, Medicine Bow National Forest, in 1919. This plot was clear-cut and burned in 1909. In the background is the virgin stand of plot 1

sentiment against extensive clear-cuttings. Where all of the trees are too old to make material additional growth, clear-cutting represents the most practical way to dispose of existing stands. On clear-cut plots very even distribution of seedlings was obtained (fig. 4), the earlier seedlings advanced rapidly in size, and new seedlings were found to be coming in in moderate numbers. Although plot 3, on which the slash was burned, has three times as many trees as plot 4, even the 3,389 trees per acre on plot 4 (Table 3) is now, at the end of 10 years, quite sufficient for all future needs. There was in this plot but one 16×16 foot section which had no seedlings upon it. In some of the denser sections there will probably be losses due to crowding.

It is regrettable that there is no means of showing the extent to which these plots have been seeded from the side. Even in plot 3, however, where much of the ground was burned over in burning the slash, it can not be doubted that many, at least of the early seedlings,

came from cones left on the ground. The reproduction occurring on several other more extensive clear-cut areas in the region leads to the belief that the slashings are quite adequate to supply all the seed needed for reasonable, or even quite heavy, reproduction. Moreover, theoretically at least, the cones left in the tops of trees will not open all at once, but gradually over several years, and some of the seedlings will surely meet favorable seasons for their ready establishment.

Since plot 4 had the benefit of all the seed from its tree tops, and plot 3 of only a portion, it is quite evident that slash left on the ground must make conditions less favorable for seedling establishment. This is thought to be due in part to the undisturbed humus layer, increased by falling needles which tend to keep seeds from reaching the more even moisture of mineral soil, and in part to the effect of shade in preventing germination. Experiment by Bates has shown that lodgepole seed germinates promptly and fully only when subjected to considerable daily fluctuations of temperature. Any factor which delays germination is quite likely to decrease its total effective quantity.

There can be no doubt, then, that the leaving of slash has a repressive effect on reproduction, which may be decidedly beneficial to the future forest in localities where reproduction is likely to be too abundant. This effect can undoubtedly be controlled quite definitely by burning more or less of the slash and of the ground area, as experience with local conditions may demand.

On the other hand, since it is known that the richness of the forest soil, particularly in nitrogen, is derived largely from organic matter, it must appear to be poor conservation, poor preparation for the growth of the next crop, to destroy either slashings or the partly decomposed material of the forest floor more than is absolutely necessary, most certainly in a region where the climate does not favor humus accumulation and soil-building.

At the outset it appeared that the seedlings on the open ground of plot 3 were developing more rapidly than those in plot 4, where in many cases the shade of undecayed small poles and branches retarded them. By 1919 many of the trees of plot 4 were gaining freedom from this obstruction and were somewhat in advance of those of plot 3. The smaller proportion of very small trees in plot 4 at this time makes its average height 2 inches greater than that of plot 3, which has actually fewer trees over 18 inches high.

To determine whether at this later stage, released from shading, the trees of plot 4 were growing any faster than those of plot 3, 20 sections of each plot were again examined in 1924. This portion of plot 3 showed a decline from 1907 to 1867 trees, or 2.1 per cent in 5 years, while plot 4 increased from 709 to 805, a gain of 13.5 per cent. Considering only the number of trees in each area which were 18 inches or over in 1919—the group least likely to have been affected by losses—it is found that the best 90 trees [8] of plot 3 advanced from an average height of 21.3 to 46.3 inches, or gained 25 inches in 5 years, while the best 98 on plot 4 gained 28 inches, or from 25.1 to 53.1 inches. This latter group might be growing faster

[8] That these are the same trees comprising the largest 90 trees in 1924 is not certain, as there is no means of identification other than size, considering each section separately.

because of having reached a more advanced stage, but if the 10 excessively large trees of plot 4 be eliminated, the average growth of the remaining 88 is still about 1.2 inches more than that of the plot 3 trees. This result is further influenced by the fact that one section of plot 4 contains 32 of the 98 trees, while the most crowded section of plot 3 contains only 12 large trees. The growth on the former was only 24.9 inches, reducing the average considerably.

It is, therefore, believed that plot 4 conditions are and will continue to be more favorable for growth because the soil has been left in better condition, in addition to the fact that this area will not be so soon handicapped by crowding.

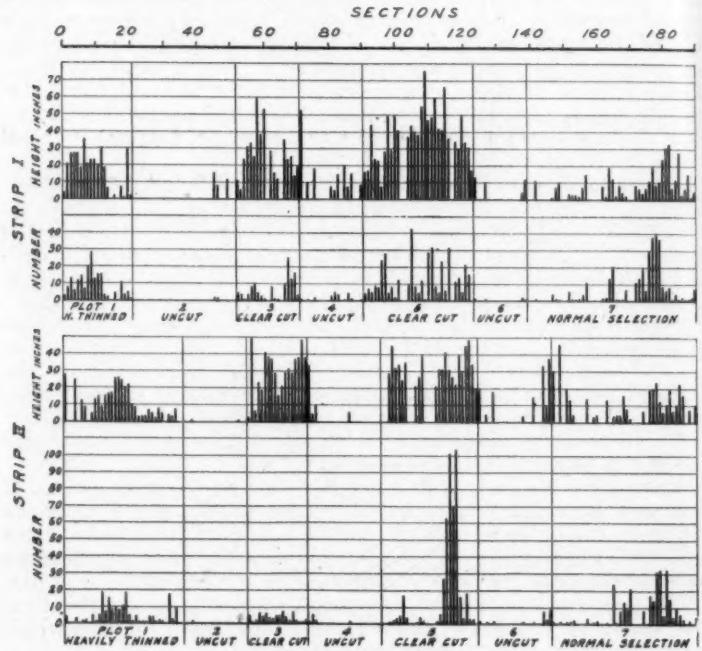

FIG. 5.—Number and height of seedlings on each section of the two observation strips of the Gunnison area, 1924

GUNNISON AREA

A summary of the progress of reproduction on this area may be found in Table 4. First, however, it will facilitate an understanding of these results to note in Figure 5 the distribution of the seedlings in 1924. This diagram records both the number and average height of the seedlings on the one hundred and ninety 10×10 foot squares of each of two sample strips. (Compare fig. 2.) It indicates that the uncut plots 2, 4, and 6 contain practically no reproduction except along their edges, adjacent to the open areas. The size of the seedlings where Strip I crosses plot 4, and where Strip II crosses plot 6, might be thought to be exceptions to this rule, but the small number

of these trees indicates that their occurrence is probably due to small openings such as might be expected in any stand.

TABLE 4.—*Average number and height of seedlings, by plots and strips, Gunnison area*

STRIP I

Plot number and method	Average light intensity *	Average number of seedlings per section				Gain from 1919 to 1924	Heights in 1924
		1913	1916	1919	1924		
						Per cent	*Inches*
Plot 1, heavily thinned	70	2.71	4.96	6.19	8.95	45	21.8
Plot 2, uncut	30	.03	.48	.16	.16	0	12.2
Plot 3, clear cut	95	1.00	4.26	4.68	6.31	35	25.7
Plot 4, uncut	51	.79	1.28	.95	1.31	38	12.3
Plot 5, clear cut	77	9.24	11.64	11.56	12.21	6	37.2
Plot 6, uncut	48	.06	.18	.12	.31	158	8.6
Plot 7, lightly thinned	51	1.51	4.60	5.12	6.25	22	11.8

STRIP II

Plot 1, heavily thinned	82	3.58	5.75	5.14	4.72	−8	14.2
Plot 2, uncut	48	.26	1.26	0	.10	∞	1.0
Plot 3, clear cut	100	1.61	3.17	3.11	3.83	23	26.4
Plot 4, uncut	43	0	.18	.09	.41	356	8.8
Plot 5, clear cut	88	13.86	14.86	16.69	15.79	−5	30.8
Plot 6, uncut	40	.18	.59	.27	1.18	337	27.6
Plot 7, lightly thinned	62	2.11	4.66	4.43	6.20	40	12.1

* Computed from one measurement on each section, made between 11 a. m. and 1 p. m., during first reproduction count in 1913. In the clear-cut strips the photometer was held at a central point, near the ground, and the readings may have been influenced by the presence of brush.

The clear-cut plots 3 and 5 contain quite uniformly large seedlings, and their distribution is satisfactory except on Strip II, plot 5, where there is considerable amassing in one small area. No good reason can be given for this, except that plot 5 as a whole was considerably disturbed after cutting by the movement of timber through it, from a large area above. In plot 3 (fig. 6) the slash was probably left more evenly scattered and the soil was stirred less. There is a slight suggestion that the excessive number of trees on plot 5 may be retarding the height growth at this early stage.

The heavily-thinned plot, on which practically no cover was left that would be of importance in shading or even in utilizing the soil space as a whole, is producing more seedlings than is clear-cut plot 3, but the seedlings of plot 3 are larger. The more lightly-cut plot 7 gives a good picture of the uneven distribution of reproduction which is likely to occur on "improvement cuttings," owing to the fact that the trees left can not be evenly spaced.

Throughout these areas, new or small suppressed seedlings are a rather inconsequential part of the whole, despite the fact that all of the plots show increases in total numbers from 1919 to 1924. There are a few such seedlings in plot 1, even more in plot 7, and some in the clear-cut plot 5 where the ground is still open; but they are practically absent from all areas there the larger seedlings are numerous, and the uncut stands in 1924 showed no such crops of probably short-lived seedlings as appear on the Medicine Bow areas.

In the clear-cut strips of this area, unlike those of Medicine Bow, there is no concentration of seedlings, either as to size or number,

adjacent to the uncut timber. This is possibly because the Gunnison area being on a north exposure, partial shading was not an added benefit to seedlings. At any rate, the abundance of seedlings in the centers of the clear-cut strips seems to verify the belief that seeding from the side is not an essential factor in the result.

Turning now to Table 5, where the data for each strip are given, it will be seen that on the uncut areas, even when the marginal regions are included, the seedlings with heights in 1924 of 1 to 28 inches average considerably less than 1 per 100 square feet, while on the clear-cut plots they average practically 1 per 10 square feet, with heights close to 30 inches. Only in plot 5, with about 7,000 per acre on Strip II, could the number possibly be considered excessive, but in both these and the thinned plots the numbers are undoubtedly

F-170593

FIG. 6.—Reproduction in 1922 on plot 3, Gunnison area. A portion of the cover is formed by brush. In the background is the virgin timber in plot 4, of somewhat smaller size than that of Medicine Bow area.

adequate. Height growth is apparently being retarded in the selection stand (plot 7), but this plot has possibly been a little slower than the others in acquiring its seedlings.

TABLE 5.—*Average number of seedlings by plots for both strips, Gunnison area, 1924*

Plot number and method	Total number of sections	Seedlings			Average per section	Average per acre
		Strip I	Strip II	Total		
Plot 1, heavy cutting	57	188	170	358	6.28	2,736
Plot 2, uncut	50	5	2	7	.14	61
Plot 3, clear-cut	37	120	69	189	5.11	2,225
Plot 4, uncut	41	25	9	34	.83	361
Plot 5, clear-cut	62	403	458	861	13.89	6,049
Plot 6, uncut	38	5	26	31	.82	355
Plot 7, selective cutting	95	319	273	592	6.23	2,714

CONCLUSIONS

Observations on two sets of plots, for 10 and 12 years, respectively, after cuttings of various kinds in typical lodgepole pine stands of better than average qualities for their respective localities, lead to the following conclusions:

Quite independent of fire, lodgepole pine reproduction is easily obtained on these medium-to-good sites whenever sufficient cutting is done to make room for it.

A very light thinning (30 to 35 per cent by volume) induces the partial establishment of great numbers of seedlings, most of which succumb in a few years; but enough are likely to remain for many years and, following a second cut, to develop quickly into trees.

Reproduction under partly cut stands may develop well in spots but is likely to be as patchy as is the stand which is left. This unevenness is further accentuated by the burning of slash in piles, which tends to remove all root competition from the immediate area.

The evidence points unmistakably to the advantages of clear cutting. Abundant reproduction was obtained on all clear-cut strips, and, it is believed, can be obtained adequately on clear-cut areas of any extent if the cones which are characteristically found in the tops of trees cut are not wholly, or largely, destroyed by burning the slash. Abundant proof of this has also been afforded on less favorable areas of considerable extent.

A further advantage is to be gained by leaving slash after clear cutting. Despite the destruction of most of the cones and seeds, the burning of a clear-cut area on the Medicine Bow produced more seedlings than where the slash was left, thus leading to the conclusion that slash has a repressive effect on reproduction, tantamount on many areas to a thinning operation where otherwise too dense a stand would be obtained. Indeed, leaving or burning may be so regulated as to insure an adequate number of seedlings and to prevent excesses. Burning may at times also be employed to lighten the stand. If the tops were especially heavy with cones, leaving all of the slash might not be sufficiently repressive, and where this danger is great, it would be a simple matter to provide for the burning of the more heavily laden tops.

It is still problematical what effect slash disposal may have on severe sites, such as south exposures, where lodgepole reproduction is sometimes sparse. It is believed, however, that the repressive effect of scattered slash, which is partly due to shading, would be slight under these conditions, and that the slash would be generally beneficial.

There is evidence that the leaving of slash is beneficial to the soil and to growth of the reproduction stand after the first few years, and it should, therefore, be left in all places where the fire hazard is not too greatly increased by its presence.

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STUDIES OF THE RELATIVE RESISTANCE TO ARSENICAL POISONING OF DIFFERENT STRAINS OF CODLING-MOTH LARVAE[1]

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INTRODUCTION

The wide variation in the requirements for the control of the codling moth (*Carpocapsa pomonella* L.) has been brought out at various conferences on arsenical residues and codling-moth control which have been held since the spring of 1926. The opinion has generally prevailed that the varying requirements were due to the different "conditions" in the different fruit-growing sections. It was understood that the "conditions" meant climate, life history of the insect, and to a less extent parasites, predators, and orchard management. The climatic factors seemed to be regarded as the most important.

This investigation was undertaken with the hope of obtaining some definite information on the influence of some of the factors by the process of their elimination. It was assumed that the direct influence of a different climate would disappear when codling-moth larvae from such widely separated States as Colorado and Virginia were subjected to the same tests in Virginia. The known seasonal history of the insect is about the same in the parts of Colorado and Virginia from which the larvae came. Furthermore, the effect of parasites and predators was eliminated as a factor in the insectary. In short, it was expected that a comparison of the larvae in the same environment would yield illuminating evidence on whether the relative difficulty of control depends (1) on such environmental factors as climate and natural enemies or (2) on differences in the larvae themselves.

Through the courtesy of George M. List, of the Colorado Agricultural Experiment Station, larvae were collected by and received from Otto M. Forry, horticulturist at Grand Junction, Colo. The larvae arrived in Winchester, Va., on April 6 and October 24, 1927. The first shipment provided the source of the western larvae used in the experiments of 1927, the results of which have already been published.[2] Descendants from the larvae of the October shipment were the source of supply of Colorado material in all experiments of 1928, excepting one test in which descendants of larvae from the two shipments were compared.

For the purpose of comparing Washington and Virginia larvae in the same environment in the State of Washington as well as in Virginia, a few Virginia larvae were sent on March 6, 1928, to E. J. Newcomer, of the Bureau of Entomology laboratory of the United States Department of Agriculture at Yakima, Wash., in exchange for a shipment of larvae from that locality which arrived on March 29.

[1] Received for publication Dec. 14, 1928; issued March, 1929.

[2] HOUGH, W. S. RELATIVE RESISTANCE TO ARSENICAL POISONING OF TWO CODLING MOTH STRAINS. Jour. Econ. Ent. 21: 325-329. 1928.

The writer is pleased to acknowledge Mr. Newcomer's cooperation and is indebted to him for voluntary permission to use his results in this paper, thus making possible a convenient comparison of the Washington and Virginia larvae in the two States. Newcomer's tests were conducted according to his own methods and independently of the work in Virginia. Full credit is due him for the results reported in this paper.

All of the Virginia larvae came from Frederick County and were reared in the insectary of the Winchester research laboratory.

METHODS

The methods used in 1928 were similar to those employed in the first three experiments in 1927. Eggs were deposited on pear

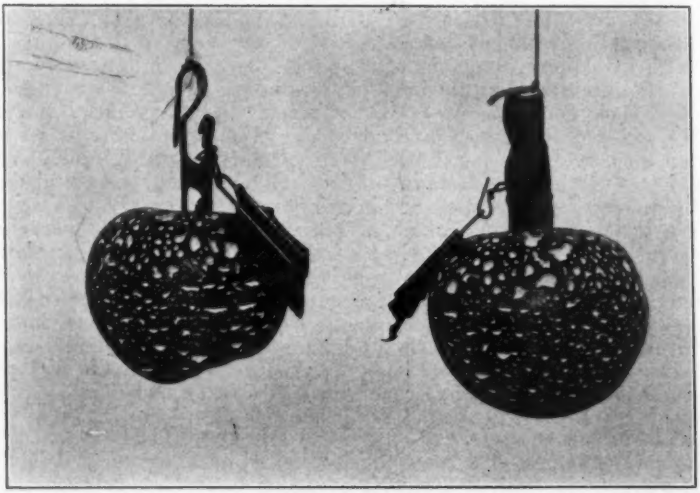

FIG. 1.—Suspended apples showing deposit of lead-arsenate spray and method of attaching pear leaves on which codling-moth eggs had been deposited

leaves which had been placed in lantern-globe breeding cages. Whenever necessary the leaves were cut into divisions so that the same number of eggs remained on each leaf section used. At the proper time the leaves or leaf sections were fastened in paper clips and suspended in such a way that the leaf rested on the side of the suspended fruit. In the fourth and fifth experiments 8 eggs were thus placed on each apple and in the sixth the number was increased to 10.

Arkansas (Mammoth Black Twig) apples were used exclusively throughout the season. All of the apples were picked from four trees which received a petal-fall spray on May 14. In this spray 3 pounds of lead arsenate and 10 quarts of lime sulphur were used in 100 gallons of water. No cover sprays were applied. Except as noted in Table 8, no attempt was made to prevent the larvae from having access to the calyx end of the apples. Most of the apples were picked on the day before the day of the experiment but a few were picked on

the same day. Each apple was suspended by attaching a suspended card holder to the stem as shown in Figure 1.

After the apples for the fourth, fifth, and sixth experiments had been suspended, lead arsenate at the rate of 4 pounds to 100 gallons of water was thoroughly applied with a bucket sprayer equipped with a Vermorel nozzle. As soon as the spray dried a second application was made to insure a very heavy and uniform distribution of all sizes of spray globules. Each application ceased when the spray dripped from the fruit.

Immediately after the spray had dried a paper clip holding a pear leaf was so suspended from the card holder, by means of a copper-wire hook, that the leaf and clip rested on the side of the apple. The fruit was not touched by any other object until time for the final examination. The eggs hatched within one to three days after they were placed on the fruit. The only exception to this method of exposing the fruit to the attack of the larvae was in experiment C of Table 8, when the newly hatched larvae were placed on the apples with the aid of a camel's-hair brush. Final examination of the fruit began five to seven days after the eggs had hatched. In each experiment the apples were examined first for the Virginia larvae and on the same or the next day they were examined for the Colorado larvae.

The fruit was sprayed and the eggs were attached for the fourth, fifth, and sixth experiments on June 8, June 22, and August 9, respectively.

EXPERIMENTAL RESULTS

STRAINS

The results given in Table 1 show that the Colorado larvae were consistently more capable of entering the sprayed apples than were the Virginia larvae. The double application of lead arsenate was quite effective in reducing the proportion of live Virginia larvae (6.4 to 1.5 per cent), but approximately one-fourth (24.7 to 29.8 per cent) of the Colorado worms not only entered the fruit but remained alive. At the time of examination they showed no ill effects from penetrating the heavily sprayed apple skin. By making a double application of the lead-arsenate spray the poison deposit on the fruit was considerably heavier than that obtained in the three experiments of 1927. A comparison of the results of the two years shows that the percentage of live larvae of each strain was slightly reduced in the season's work of 1928, but the Colorado larvae consistently demonstrated a marked superiority over the Virginia larvae in their ability to enter the sprayed fruit. Furthermore, it will be seen (Table 1, experiment 4) that the Colorado larvae which came from ancestors living under Virginia climatic conditions since April 6, 1927, were equally as successful as those whose parents passed only the winter in Virginia. Descendants from the latter group were used in tests succeeding the fourth experiment.

TABLE 1.—*Comparative tests with Colorado, Washington, and Virginia codling-moth larvae on apples which received two applications of lead-arsenate spray diluted at the rate of 4 pounds of lead arsenate to 100 gallons of water, Winchester, Va., 1928*

Experiment No.	Brood and strain of larvae	Eggs hatched (number)	Larvae found alive				Total larvae found alive	
			In side		In calyx			
			Number	Per cent	Number	Per cent	Number	Per cent
4	First brood:							
	Colorado [a]	681	158	23.2	15	2.2	173	25.4
	Colorado [b]	446	106	23.7	13	2.9	119	26.6
	Virginia	757	5	.6	4	.5	9	1.1
	Washington	881	42	4.7	7	.8	49	5.5
	Virginia (check)	470	240	51.0	13	2.7	253	53.8
5	First brood:							
	Colorado	730	168	23.0	13	1.7	181	24.7
	Virginia	646	1	.1	2	.3	3	.4
	Washington	680	32	4.7	10	1.4	42	6.1
	Colorado (check)	398	272	68.3	27	6.8	299	75.1
	Virginia (check)	293	177	60.4	9	3.0	186	63.4
6	Second brood:							
	Colorado	928	243	26.2	34	3.6	277	29.8
	Virginia	974	6	.6	9	.9	15	1.5
	Colorado (check)	438	344	78.5	25	5.7	369	84.2
	Virginia (check)	480	271	56.4	24	5.0	295	61.4

[a] Parent individuals from Colorado on Oct. 24, 1927.
[b] Ancestors from Colorado on Apr. 6, 1927, and reared through 2 generations in 1927.

The Washington larvae were much less capable of entering the sprayed fruit than the Colorado worms. In both tests they appeared more like the Virginia larvae, although they consistently demonstrated a superiority over the native strain. The percentage of surviving Washington larvae (Table 1) and the percentage of surviving larvae of the Colorado-Virginia crosses (Table 5) show an interesting similarity.

TABLE 2.—*Injuries caused by Colorado, Washington, and Virginia codling-moth larvae on the sides of apples which received two applications of lead-arsenate spray diluted at the rate of 4 pounds of lead arsenate to 100 gallons of water, Winchester, Va., 1928*

Experiment No.	Brood and strain of larvae	Eggs hatched (number)	Side entrances		Side stings		Total side injuries	
			Number	Per cent	Number	Per cent	Number	Per cent
4	First brood:							
	Colorado [a]	681	158	23.2	224	32.9	382	56.1
	Colorado [b]	446	106	23.7	104	23.3	210	47.0
	Virginia	757	5	.6	113	14.9	118	15.5
	Washington	881	42	4.7	251	28.5	293	33.2
	Virginia (check)	470	240	51.0	12	2.6	252	53.6
5	First brood:							
	Colorado	730	168	23.0	211	28.9	379	51.9
	Virginia	646	1	.1	87	13.5	88	13.6
	Washington	680	32	4.7	138	20.3	170	25.0
	Colorado (check)	398	272	68.3	12	3.0	284	71.3
	Virginia (check)	293	177	60.4	16	5.4	193	65.8
6	Second brood:							
	Colorado	928	243	26.2	287	30.9	530	57.1
	Virginia	974	6	.6	238	24.4	244	25.0
	Colorado (check)	438	344	78.5	14	3.2	358	81.7
	Virginia (check)	480	271	56.4	37	7.7	308	64.1

[a] Parent individuals from Colorado on Oct. 24, 1927.
[b] Ancestors from Colorado on Apr. 6, 1927, and reared through 2 generations in 1927.

The relative number of side injuries (Table 2) caused by the Colorado, Washington, and Virginia larvae rank in the order named. In order to allow ample time for the larvae to demonstrate clearly whether the injury caused was to be classed as a "sting" or a successful "entrance," a minimum of five days was allowed between the time of hatching of the egg and examination of the injury. Quite a number of the stings caused by the Colorado larvae were deep, indicating that the larvae lived a day or more after penetrating the skin. To a slightly less extent this was also true of the Washington larvae. Deep stings were not as common on the fruit attacked by the Virginia worms. A comparison of the number of injuries caused by the Washington larvae (Table 2) and the Colorado-Virginia crosses (Table 6) emphasizes the similarity of their performance on the sprayed fruit.

Newcomer,[3] working at Yakima, Wash., compared Washington and Virginia larvae of the first brood, spraying the fruit once with lead arsenate diluted at the rate of 2 pounds to 100 gallons of water. Just prior to spraying, the calyx end of the apples was filled with shellac. Five tests were made. In the first of these four worms were placed on each apple, and in the succeeding tests 10 worms were placed on each. The results of Newcomer's experiments (Table 3) show a slight superiority of the Washington larvae. The same table shows results obtained at Winchester with second-brood Colorado and Virginia larvae on fruit sprayed once with lead arsenate diluted at the same rate.

TABLE 3.—*Comparative tests with Colorado, Washington, and Virginia codling-moth larvae on apples which received a single application of lead-arsenate spray diluted at the rate of 2 pounds of lead arsenate to 100 gallons of water*

ON JONATHAN APPLES AT YAKIMA, WASH.,[a] 1928

Brood and strain of larvae	Number of larvae used	Live larvae found				Total live larvae	
		In side		In calyx[b]		Number	Per cent
		Number	Per cent	Number	Per cent		
First brood:							
Virginia	220	36				36	16.4
Washington	220	49				49	22.3
Washington (check)	170	99				99	58.2

ON ARKANSAS (MAMMOTH BLACK TWIG) APPLES AT WINCHESTER, VA., 1928

Experiment No.	Brood and strain of larvae	Eggs hatched (number)	Live larvae found				Total live larvae	
			In side		In calyx		Number	Per cent
			Number	Per cent	Number	Per cent		
6a	Second brood:							
	Colorado	350	175	50.0	20	5.7	195	55.7
	Virginia	331	34	10.2	6	1.8	40	12.0
6b	Second brood:							
	Colorado	234	112	47.8	13	5.6	125	53.4
	Virginia	239	31	12.9	4	1.7	35	14.0

[a] Summary of 5 tests made by E. J. Newcomer.
[b] Calyx cup of Jonathan apples covered with shellac to prevent entrance through the calyx end.

[3] NEWCOMER, E. J. Letter of July 20, 1928. Used by permission.

Experiments 6a and 6b ran simultaneously with experiment 6 of Table 1 and show an increase in the percentage of surviving larvae when a weaker arsenical coating protected the fruit. The results of experiments 6, 6a, and 6b agree with the conclusions of Smith,[4] "that the degree of protection varies directly with the concentration of the spray and with the amount of lead arsenate on the fruit." The percentage of surviving Virginia larvae at Yakima (16.4 per cent) and at Winchester (12 and 14.6 per cent) reveals no important difference in the performance of this strain in the two States. Reference to Table 4 shows that the Virginia larvae caused about the same proportion of side injuries at Yakima and Winchester. The Colorado larvae, however, continued to demonstrate a marked superiority over the Virginia larvae when allowed to attack apples sprayed with the weaker concentration of lead arsenate, as indicated in experiments 6a and 6b. It will be seen that the percentage of injuries caused by the former on the sprayed fruit (70.2 and 62.8 per cent in Table 4) was equal to the percentage of injuries (64.1 per cent in experiment 6, Table 2) caused by Virginia larvae which were attacking unsprayed fruit at the same time. Furthermore, the percentage of surviving Colorado larvae in the sprayed fruit (55.7 and 53.4 per cent in Table 3) and the percentage of live Virginia larvae (61.4 per cent in experiment 6, Table 1) in the unsprayed fruit did not differ greatly.

TABLE 4.—*Injuries caused by Colorado, Washington, and Virginia codling-moth larvae on the sides of apples which received a single application of lead-arsenate spray diluted at the rate of 2 pounds of lead arsenate to 100 gallons of water*

ON JONATHAN APPLES AT YAKIMA, WASH., 1928 [a]

Brood and strain of larvae	Larvae used (number)	Side entrances		Side stings		Total side injuries caused by larvae	
		Number	Per cent	Number	Per cent	Number	Per cent
First brood:							
Virginia	220	36	16.4	46	20.9	82	37.2
Washington	220	49	22.3	58	26.3	107	48.6
Washington (check)	170	99	58.2	8	4.7	107	62.9

ON ARKANSAS (MAMMOTH BLACK TWIG) APPLES AT WINCHESTER, VA.

Experiment No.	Brood and strain of larvae	Eggs hatched (number)	Side entrances		Side stings		Total side injuries caused by larvae	
			Number	Per cent	Number	Per cent	Number	Per cent
6a	Second brood:							
	Colorado	350	175	50.0	71	20.2	246	70.2
	Virginia	331	34	10.2	101	30.5	135	40.7
6b	Second brood:							
	Colorado	234	112	47.8	35	15.0	147	62.8
	Virginia	239	31	12.9	62	26.0	93	38.9

[a] Summary of 5 tests made by E. J. Newcomer. Calyx cup of Jonathan apples covered with shellac to prevent entrance through calyx end.

CROSSES

Matings of Colorado and Virginia moths were made with both the spring-brood and first-brood moths. The first generations of these

[4] SMITH, R. H. AN INVESTIGATION OF SPRAY COVERAGES AND ARSENICAL RESIDUE IN RELATION TO THE CONTROL OF THE CODLING MOTH. Jour. Econ. Ent. 21: 571-588, illus. 1928.

crosses were very much alike (Table 5) in their ability to enter the heavily sprayed fruit. The percentages of side injuries (Table 6) also show a similarity in the accomplishments of the crosses. The F_1 generations were superior to the Virginia larvae in producing side injuries and entering the fruit, but they were distinctly inferior to the Colorado larvae in these respects, as a study of Tables 1, 2, 5, and 6 will show. Similar results were obtained in 1927 in comparative tests with parent strains and the F_1 generations.

TABLE 5.—*Comparative tests with the F_1 and F_2 generations of the crosses of the Colorado and Virginia codling moths on apples which received two applications of lead-arsenate spray diluted at the rate of 4 pounds of lead arsenate to 100 gallons of water, Winchester, Va., 1928*

Experiment No.	Cross and generation	Eggs hatched (number)	Larvae found alive				Total larvae found alive	
			In side		In calyx		Number	Per cent
			Number	Per cent	Number	Per cent		
4	Colorado ♂ × Virginia ♀ F_1	720	37	5.1	11	1.5	48	6.6
	Colorado ♀ × Virginia ♂ F_1	416	19	4.5	2	.5	21	5.0
6	Colorado ♂ × Virginia ♀ F_1	898	44	4.9	13	1.4	57	6.3
	Colorado ♀ × Virginia ♂ F_1	624	32	5.1	13	2.1	45	7.2
4	Colorado ♂ × Virginia ♀ F_2	735	42	5.7	11	1.5	53	7.2
	Colorado ♀ × Virginia ♂ F_2	705	18	2.5	7	1.0	25	3.5
5	Colorado ♂ × Virginia ♀ F_2	629	17	2.7	1	.1	18	2.8
	Colorado ♀ × Virginia ♂ F_2	663	9	1.3	3	.5	12	1.8
6	Colorado ♂ × Virginia ♀ F_2	974	62	6.4	16	1.6	78	8.0
	Colorado ♀ × Virginia ♂ F_2	874	27	3.0	14	1.6	41	4.6

TABLE 6.—*Injuries caused by the young larvae of the F_1 and F_2 generations of the crosses of Colorado and Virginia codling moths on the sides of apples which received two applications of lead-arsenate spray diluted at the rate of 4 pounds of lead arsenate to 100 gallons of water, Winchester, Va., 1928*

Experiment No.	Cross and generation	Eggs hatched (number)	Side entrances		Side stings		Total side injuries caused by larvae	
			Number	Per cent	Number	Per cent	Number	Per cent
4	Colorado ♂ × Virginia ♀ F_1	720	37	5.1	166	23.0	203	28.1
	Colorado ♀ × Virginia ♂ F_1	416	19	4.5	88	21.2	107	25.7
6	Colorado ♂ × Virginia ♀ F_1	898	44	4.9	210	23.3	254	28.2
	Colorado ♀ × Virginia ♂ F_1	624	32	5.1	137	21.9	169	27.0
4	Colorado ♂ × Virginia ♀ F_2	735	42	5.7	153	20.8	195	26.5
	Colorado ♀ × Virginia ♂ F_2	705	18	2.5	134	19.0	152	21.5
5	Colorado ♂ × Virginia ♀ F_2	629	17	2.7	80	12.7	97	15.4
	Colorado ♀ × Virginia ♂ F_2	663	9	1.3	41	6.2	50	7.5
6	Colorado ♂ × Virginia ♀ F_2	974	62	6.4	272	27.9	334	34.3
	Colorado ♀ × Virginia ♂ F_2	874	27	3.0	231	26.4	258	29.5

A small wintering brood of larvae of the F_1 generation of each cross made in 1927 provided the parents of the F_2 generations for experiment 4 of Table 5. The F_2 generations used in experiments 5 and 6 came from matings made in the spring. It will be seen (Tables 5 and 6) that the performance of the F_2 generations was not strikingly different from that of the F_1. There was, however, a tendency for the second generation of the Colorado male-Virginia female cross to appear somewhat superior to the Colorado female-Virginia male cross. In experiment 5 both crosses in the F_2 generation sank to a low level in their capacity to enter and injure the fruit. The reason for this is not entirely clear. The young larvae for this experiment hatched from eggs which had been subjected to a cold-storage temperature of approximately 32° F. for two weeks in order to prevent hatching before time for the experiment. The embryos were in various stages of development when placed in cold storage. Perhaps the vitality of the larvae was reduced by prolonging the incubation period of the eggs to 21 and 25 days. The normal incubation period at this time was 8 and 9 days. On a number of occasions the length of the egg stage has been extended from a normal incubation period of 6 to 8 days to 14 to 16 days without any apparent effect on the vitality of the young worms. Usually, however, the embryos had not developed very far when the eggs were placed in cold storage.

POSSIBLE DIFFERENCES BETWEEN VIRGINIA AND COLORADO STRAINS

Codling-moth larvae normally cast aside bits of apple tissue as they penetrate the fruit. They do not appear to eat much until they have buried themselves beneath the discarded pellets. It was thought that a difference in the habit of casting aside or consuming the outer tissue might account for the success of one strain and the failure of another in surviving an attack on sprayed fruit. But no such difference has been observed in comparing Colorado and Virginia larvae. However, it was thought that the difference might be so slight as to pass unobserved and, therefore, more information was desired on its probable importance.

Comparative tests were made with young unstarved larvae, which habitually throw aside most, if not all, of the first tissue attacked, and young larvae starved long enough to be much less persistent in rejecting the first bits of tissue torn loose. With a fine-pointed scalpel, small pits, about 1 c. mm. in size, were cut in the sides of apples. One drop of a solution of arsenic acid (1gm. As_2O_5 in 1,000 ml. water) was placed in each pit. By measuring the size of each drop as it formed on the end of a microinjection needle it was possible to obtain drops of approximately the same volume (0.001 ml.) for each pit. A larva was placed in each pit as soon as the pit was sufficiently dry. Some of the larvae were taken from hatching jars and starved in stender dishes for minimum periods of 6½ to 8 hours prior to the experiment. Others were transferred directly from the hatching jars to the pits. The unstarved worms always began their attack by tearing off and rejecting small bits of apple tissue. The starved larvae, on the other hand, were noticeably less persistent in this respect. They were more intent on satisfying their hunger. Although the starved larvae cast aside bits of the apple tissue at irregu-

lar intervals, they rejected less of the tissue at the beginning of the attack than the unstarved larvae.

The pits were examined 40 hours after the larvae had been placed in them, and the results are recorded in Table 7. It is apparent that the starved larvae of each strain were less successful in penetrating poisoned apple tissue than were the unstarved larvae of the same strain. It is also apparent that the unstarved Virginia worms were not so impressively superior to the starved Colorado larvae in the percentage (16.2 and 11.2 per cent, respectively) of surviving individuals. The results were surprising to the writer, since the unstarved Virginia larvae appeared to reject distinctly more of the first tissue attacked than did the starved Colorado larvae.

For the purpose of observing the work done by the larvae in penetrating unsprayed fruit, newly hatched worms of the Colorado and Virginia strains were placed on sound apples which had the calyx end coated with paraffin. Each larva was observed from the time it began its attack on the apple skin until a pit was made into which it crawled and closed the entrance with frass. The average time consumed by 111 Colorado larvae was 87.3 minutes, minimum 33, and maximum 195 minutes. The average time consumed by 107 Virginia larvae was 91.8 minutes, minimum 13, and maximum 224 minutes. Twenty-one Colorado larvae and 12 Virginia larvae buried themselves in the side of the apples in less than an hour. But 18 Colorado larvae and 15 Virginia larvae required two hours or more to enter the same apples. During the time of making the observations no consistent difference was noted between the larvae of the two strains with respect to energy expended, speed in working, or habits of attack.

TABLE 7.—*Results of feeding experiments with young, starved, and unstarved Colorado and Virginia codling-moth larvae on apple tissue poisoned with a solution of arsenic acid, 1 gram of anhydrous As_2O_5 in 1,000 ml. of water, Winchester, Va., 1928*

Experiment No.	Strain	Starved larvae		Unstarved larvae		Experiment No.[a]	Check experiments	
		Number in experiment	Number alive after 40 hours	Number in experiment	Number alive after 40 hours		Number of larvae in experiment	Number of larvae alive after 40 hours
1	Colorado	10	0	10	5	a	10	9
	Virginia	10	0	10	5		10	8
2	Colorado	10	2	10	0	b	15	15
	Virginia	10	0	10	1		8	8
3	Colorado	10	0	10	2	c	10	10
	Virginia	10	0	10	0		10	9
4	Colorado	10	1	10	2			
	Virginia	10	0	10	0			
5	Colorado	10	1	10	4			
	Virginia	10	1	10	2			
6	Colorado	10	2	10	7			
	Virginia	10	0	10	2			
7	Colorado	10	1	10	5			
	Virginia	10	0	10	2			
8	Colorado	10	2	10	1			
	Virginia	9	0	10	1			
Total	Colorado	80	9	80	26		35	34
	Virginia	79	1	80	13		28	25
Per cent	Colorado		11.2		32.5			97.1
	Virginia		1.2		16.2			89.2

[a] Experiments a, b, and c are check experiments in which the larvæ were placed in unpoisoned pits on the apples.

It was thought that perhaps the Colorado larvae might be more capable of clinging to the sides of the apples, hence fewer would be lost by dropping from the fruit. Three experiments were made in order to obtain information on this subject. Small pans containing kerosene were placed beneath unsprayed apples which had been suspended in the usual manner. In experiments A and B (Table 8) pear leaves containing eggs were attached in the usual manner. In experiment C a camel's-hair brush was used to place newly hatched larvae on the fruit. The larvae which fell from the fruit while wandering over the apples or while attempting to penetrate the skin were caught in the pans. It is obvious from Table 8 that there was not a marked difference between the strains, except in the last experiment, when the number of individuals included in the test was considerably reduced. But in the light of the three experiments it appears that the Colorado larvae demonstrated a slight superiority over the Virginia larvae in their ability to remain on the fruit. Of the 1,220 Colorado larvae in the three experiments, 44, or 3.6 per cent, dropped. Of the 1,342 Virginia larvae, 93, or 6.9 per cent, dropped. But a total of 1,002, or 82.1 per cent, of the Colorado larvae entered the apples and remained alive, as compared to 955, or 71.1 per cent, of the Virginia larvae. The slight difference in the proportion of larvae lost through dropping was not sufficient to account for the difference in the ability of the strains to enter the fruit successfully.

TABLE 8.—*Results of experiments to determine the number of Colorado and Virginia codling-moth larvae which fell from the sides of unsprayed fruit during their period of wandering and attempting to enter the apples,[a] Winchester, Va., 1928*

Experiment No.	Strain	Eggs hatched (number)	Larvae dropped		Live larvae in sides of fruit		Stings caused by larvae (number)	Total side injuries caused by larvae	
			Number	Per cent	Number	Per cent		Number	Per cent
A	Colorado	518	22	4.2	403	77.7	18	421	81.2
	Virginia	633	31	4.8	417	65.8	36	453	71.5
B	Colorado	510	12	2.3	457	89.6	20	477	93.5
	Virginia	516	30	5.8	406	78.7	28	434	84.1
C	Colorado	192	10	5.2	142	73.9			
	Virginia	193	32	16.5	132	68.3			

[a] Calyx cup of all apples covered with paraffin.

In the three experiments of Table 8, as well as in the checks of other experiments (Tables 1 and 7), the Colorado larvae were more capable than the Virginia larvae of entering unsprayed fruit and remaining alive. At the same time the Colorado larvae consistently caused more side injuries on the unsprayed fruit. While the difference in the performance of the two strains is not nearly so marked on unsprayed fruit as on sprayed fruit, it is evident that they are not equal in capacity to injure and enter the apples.

A number of feeding experiments have already been made, using solutions of arsenic acid varying in concentration from 1 gm. of $As_2 O_5$ in 400 ml. of water to 10,000 ml. of water. Although 38 tests have been completed in which 382 larvae of each strain were used, conclusions must await further experimentation. It is evident, however, that there is a correlation between the dilution of arsenic acid

used in poisoning the pits and the percentage of surviving larvae. Starved larvae have been used exclusively, and the results show a consistently higher percentage of Colorado larvae surviving than Virginia larvae. At the weaker dilutions the percentage of surviving larvae indicates an approximation of the ratios obtained in the checks, while at the higher concentrations the comparative ability to survive is more in favor of the Colorado larvae.

SUMMARY AND CONCLUSIONS

During the past two seasons tests were made comparing 4,658 Colorado and 4,229 Virginia codling-moth larvae on apples sprayed with lead arsenate, and 2,056 Colorado and 3,310 Virginia larvae on unsprayed fruit. The results show that there is a distinct difference in the ability of Colorado and of Virginia larvae to enter sprayed fruit. The former demonstrated consistently a marked superiority over the latter in the proportion of surviving larvae and also in the number of obvious side injuries produced. Similar but less marked superiority of the Colorado larvae was shown on unsprayed fruit.

At the same time experiments with 9,062 larvae of the Colorado-Virginia crosses showed that the crosses are different from either of the parents in that they are much less capable of successful penetration of sprayed fruit than Colorado larvae but somewhat more successful than Virginia larvae. In the experiments of 1928 the F_2 generations were not clearly different from the F_1 generations. There was, however, an indication of a possibly slight superiority of the cross between the Colorado males and Virginia females over the reverse cross in the second generation.

Comparative tests with 1,461 larvae whose parents came from Yakima, Wash., indicate that Washington codling-moth larvae are similar to the Colorado-Virginia crosses in their ability to enter and injure sprayed fruit. They were considerably inferior to the Colorado larvae and slightly superior to the Virginia larvae. Their superiority over Virginia larvae was demonstrated in comparative tests made by E. J. Newcomer at Yakima, Wash., and by the writer at Winchester, Va. When allowed to attack apples sprayed with lead arsenate diluted at the rate of 2 pounds of lead arsenate to 100 gallons of water, the Virginia larvae were not widely different in their performance at Yakima and Winchester.

In the light of the information obtained it appears reasonably certain that there are different strains of the codling moth with respect to ability to enter and injure apples sprayed with an arsenical poison. What is the nature of the difference and how did such strains originate? All attempts to discover morphological differences have failed. If there is a difference in the habit of attacking the fruit, efforts to detect it during the past two seasons have failed. The slightly greater number of Virginia larvae which fell from unsprayed fruit was not sufficient to account for the demonstrated difference between the Colorado and Virginia strains. The evidence indicates that the essential difference is within the larvae. Whatever may subsequently be learned concerning the exact nature of the difference, it is evident that the superiority or inferiority of a strain is transmitted to the next generation, and that the progeny from crossing two strains

differs from either parent through at least two generations in resistance to control by an arsenical spray.

Attention has been called [5] to the variation in the arsenical spray program required for codling-moth control in the Grand Valley of Colorado and the Shenandoah Valley of Virginia. The number of arsenical sprays recommended in Washington [6] is intermediate between that called for by the Colorado and Virginia programs. Spraying requirements in the apple districts of the three States from which the larvae came, rank in the same order that their respective codling-moth strains occupied in demonstrating their relative ability to enter and injure sprayed apples. Perhaps the efforts made to control the insect with arsenicals has been carried on with sufficient intensity and long enough to result in the artificial selection of the more hardy individuals which now exist as superior strains. No one will deny that the codling moth is living in a more poisonous environment than it was compelled to dwell in 20 years ago. Furthermore, of the three apple districts from which the larvae came, heavy arsenical spraying in the Grand Valley of Colorado clearly antedates that of the other two districts. At the same time the general practice of applying cover sprays for the control of the codling moth is more recent in Virginia than in either of the other districts.

Another assumption is that the superior strains originated independently of the spraying and that the increased necessity of arsenicals is the result of rather than the cause of their origin.

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[5] HOUGH, W. S. Op. cit.

[6] JOHNSON, E. C., GRINER, J. I., HARTER, G. E., NEWCOMER, E. J., NIELSEN, F. C., SPULER, A., WEBSTER, R. L., MAGNESS, J. R., ARMSTRONG, M. D., and BROWN, W. P. WASHINGTON SPRAY RECOMMENDATIONS FOR 1928. Better Fruit 22 (9): 14. 1928.

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